MONOGRAPH ON NICKEL VOL II
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Monograph on Nickel-Volume 2 7/31/74

VOLUME 2

GRAS MONOGRAPH SERIES

NICKEL

(COPIES OF ARTICLES CITED IN MONOGRAPH SUMMARY)

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

JULY 31, 1974

This publication was prepared under Contract Number FDA 72-100 with the Public Health Service, Food and Drug Administration, Department of Health, Education, and Welfare

prepared by

Tracor Jitco, Inc.

J. A. M. A. 102:1204-1206 (1934)

NICKEL CARBONYL POISONING

REPORT OF A CASE

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Intoxication with nickel carbonyl has been of rare occurrence. A number of cases that occurred in England, where the Mond process for obtaining pure nickel was first used, were reported some years ago.[1] Richter[2] reported a case of poisoning with metallic nickel, with recovery. Gron[3] reviewed the subject of nickel rash and reported a case. Stewart[4] recently has reported a case of dermatitis due to nickel and cobalt.

Armit[5] some years ago studied the toxicity of nickel carbonyl and other nickel compounds on experimental animals and found that the nickel was the toxic element and not the carbon monoxide formed. The amount of carbon monoxide liberated on dissociation from a minimal lethal dose of nickel carbonyl was sufficient to give only a 5 per cent hemoglobin saturation, which is well below the lethal level for carbon monoxide.

Nickel carbonyl is prepared by passing a current of carbon monoxide over finely divided metallic nickel. A gaseous compound is formed with the composition of $Ni(CO)_4$. The gas can be condensed into a mobile liquid, which boils at 43 C. and volatilizes at room temperature.

Characteristic changes found in the cases reported were hemorrhages, especially in the white matter of the brain and in the lungs, fatty degeneration of the walls of blood vessels, and edema of the lungs. In experimental animals hemorrhages were always found in the lungs, in the suprarenal glands in more than 50 per cent of cases and frequently in the brain. At

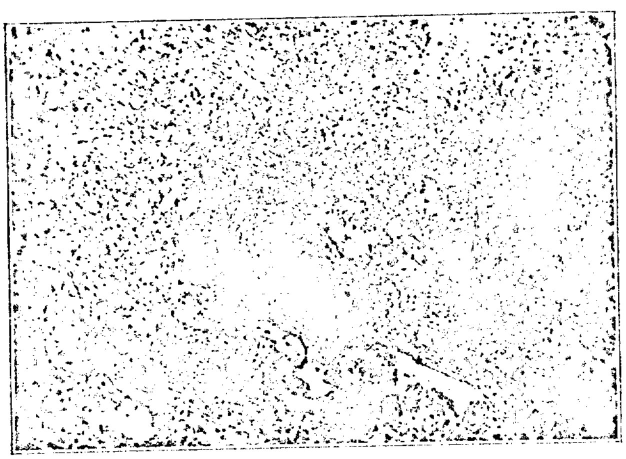

Fig. 1.—Area of lung demonstrating the degenerative and desquamative changes in the alveoli. Also mild infiltration with neutrophils and mononuclear cells. Hematoxylin and eosin stain.

times they were present in all organs. In the presence of moisture and at body temperature the inhaled nickel carbonyl is dissociated, and finely divided particles of nickel are deposited on the respiratory epithelium and are absorbed by the blood stream, causing degenerative changes in the vessel walls. The nickel is gradually removed from the lungs and some of it, at least, is excreted by the kidney, since it has been found in the urine.

The outstanding clinical features were a transient malaise with rapid recovery when brought into fresh air. At times nausea and vomiting occurred early. After from twelve to thirty-six hours cyanosis and dyspnea developed and progressively increased in

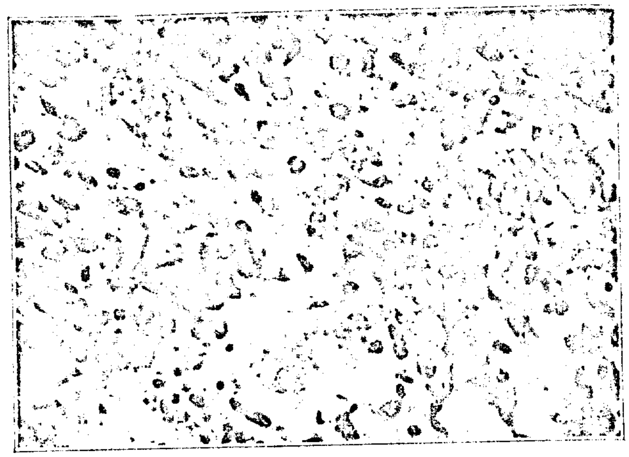

Fig. 2.—Same area of lung as in figure 1 under high power. Disintegration, fusion and proliferation of alveolar epithelium are seen more clearly. Hematoxylin and eosin stain.

severity. A productive cough with blood-stained sputum was frequently observed. Death occurred between the fourth and eleventh days.

REPORT OF CASE

C. B.,[6] a white man, aged 49, complained of dyspnea, orthopnea, nausea, vomiting, insomnia, pain in the right side of the thorax, headache and a metallic taste in the mouth. A chemist by profession, he had been working on a problem of developing a method of "cracking gasoline," in which process nickel carbonyl was used. The present trouble developed five days previous to admission to the hospital. At that time he had poured nickel carbonyl from one container to another and immediately thereafter experienced some dyspnea, from which he recovered after a brief interval. About twenty-four hours later, dyspnea recurred. He became orthopneic and cyanotic, so that at times he would "turn black." By the fourth day he became nauseated; vomiting occurred, which became increasingly severe, so that very little food could be retained. Several of his associates had mild attacks of dyspnea but quickly and completely recovered.

The physical examination revealed marked cyanosis and great respiratory difficulty. The pupils reacted normally. The tongue had a grayish coating. A firm nodular movable mass 4 by 5 cm. was palpated on the right side of the neck below the ear. Expansion of the right side of the thorax was limited, and moist râles could be heard in the middle and lower lobes anteriorly and posteriorly. Tactile and vocal fremitus were increased in these areas. The heart was within normal limits, the pulse was regular and the rate was 100. The liver edge could be felt 4 cm. below the costal border. It was slightly tender. The extremities were normal, except that the reflexes were hyperactive.

Laboratory reports revealed that the blood count was 5,100,000 red cells, hemoglobin (Tallqvist) 100 per cent, and the white cell count 15,800, with 92 per cent polymorphonuclears, of which 21 per cent were band forms. The plasma carbon dioxide combining power was 60.5 volumes per cent. Sputum was blood streaked and tenacious in character. The urine contained a few hyaline casts and a trace of albumin.

From the Department of Pathology, Baylor University College of Medicine.

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2. Richter, George: A Case of Poisoning by Metallic Nickel, J. A. M. A. 49:1696 (Nov. 9) 1907.
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4. Stewart, S. G.: Inherent Sensitivity of the Skin to Nickel and Cobalt (Allied Elements in Group VIII, Periodic System), Arch. Int. Med. 51:427 (March) 1933.
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6. Patient of Dr. Lee Hudson.

The patient had great respiratory difficulty, was very restless, became comatose and died on the seventh day after exposure to the nickel carbonyl.

At necropsy there was slight icterus of the sclerae and a faint greenish yellow tinge to the skin of the face, neck and anterior thorax. The lips and oral mucosa were deeply cyanotic. Below the right ear was a nodular mass 3 by 5 cm. that was freely movable. In the skin of the back were numerous dull reddish areas from 1 to 5 mm. in diameter. The pleural cavities contained about 15 cc. of clear fluid; their linings were everywhere smooth and glistening. Numerous dark red areas measuring from less than 1 to 5 mm. in diameter could be seen through the pleurae of both lungs. The left lung was voluminous and heavy, a reddish gray, and mottled with numerous darker red areas scattered throughout. The main bronchus and larger branches contained a frothy reddish fluid, and the lining was bluish red. Large amounts of blood-stained fluid escaped from the sectioned surfaces of the lungs. The greater portion of the left lung was noncrepitant. The right lobe similarly was heavy and dark reddish gray. The cut surfaces were very wet. The greater portion of this lung was also noncrepitant. The pericardial cavity contained about 15 cc. of clear fluid, and the lining was smooth and glistening. The right heart chambers were distended with partially clotted dark venous blood. The endothelial surfaces were smooth throughout. The apex of the left ventricle was slightly sacculated. Several small plaques of yellowish thickening were present in the bases of the aortic and mitral valve cusps. The myocardium was grayish red with small yellowish red and dark red widely scattered areas in it. The heart weighed 480 Gm. The proximal 2 cm. of the right coronary artery showed a diffuse yellow thickening of the intima with slight narrowing of the lumen. The first portion of the aorta had small areas of yellowish thickening of the intima, which increased to a moderate degree along the course of the vessel.

The liver weighed 2,100 Gm.; its capsule was smooth and the edges were slightly rounded. On sectioned surface the lobular markings were accentuated. The peripheral portions

Fig. 3.—Demyelinated areas in the base of the brain. One has a vessel in the center. Weil's myelin sheath stain.

of the lobules were yellowish gray. The kidneys together weighed 380 Gm. The surfaces, after stripping of the capsules, were finely granular. The cortex was of average width. The pyramids were dark red. The spleen was slate blue and its capsule was smooth. Sectioned surface revealed scattered throughout irregular dark red areas. Other viscera showed general passive hyperemia and small widely scattered areas of hemorrhage.

No changes were seen in the dura. The spinal fluid was clear. The meningeal veins were prominent. The dural sinuses contained dark venous blood and had smooth linings. The cerebral ventricles were not noticeably dilated. They contained a few cubic centimeters of faintly blood-tinged fluid. On multiple transverse sections numerous dark red areas were present, most numerous in the white matter, especially of the corpus callosum. They varied in size from those barely visible to a centimeter in greatest dimension. The majority were dark red; a few were brownish red. Some were elongated in outline. These small areas could be seen in the pons and the basal nuclei, and several small indefinite darker grayish areas were present in the medulla.

Fig. 4.—Higher magnification to show degenerative changes in ganglion cells and neuronophagia. Cresyl violet stain.

Histologic examination revealed that the changes in the lungs and brain were most marked and most important in this case. Sections taken from the noncrepitant areas in the lungs (fig. 1) revealed a marked edema, hyperemia, multiple hemorrhages and marked changes in the cells lining the alveoli. A slight to moderate infiltration with polymorphonuclear cells was seen. The alveoli were distorted and many to most of the lining cells had become desquamated. Many were markedly swollen and had granular cytoplasm, and others showed fragmentation. Some of the alveoli contained large cytoplasmic masses with multiple nuclei and nuclear fragments (fig. 2). These masses seemed to be fused degenerated lining cells. Some of the alveolar epithelial cells stained deeply, and miotic figures were fairly frequent in some areas. A number of small blood vessels contained fibrin thrombi in their lumens, and their walls were indistinct. There was a large amount of granular vacuolated precipitated albuminous material in the interstitial tissues and in the alveoli. The epithelium of the bronchi and bronchioles also showed degenerative changes. They were swollen and some desquamation had occurred. Many of the mucosal glands were distended with secretion.

Histologic examination of the brain revealed multiple small hemorrhages scattered throughout but most numerous in the white substance. The majority were small and in perivascular areas. Aside from the hemorrhages, multiple small areas of degeneration were scattered throughout, which showed demyelinization with a myelin sheath stain (fig. 3). These at times were about capillaries and arterioles. In some of these areas, moderate numbers of polymorphonuclear and large mononuclear cells could be seen. The hemorrhages varied somewhat in that in some the red blood cells were intact, whereas in others they were partly disintegrated. In a fair number of capillaries and arterioles, hyaline and fibrin thrombi were seen. The walls of some of the arterioles were indistinct and more or less homogeneous and hyaline-like in structure. Ordinary fat stain did not show an increase in fat. With cresyl violet stain, degenerative changes were well demonstrated in many ganglion cells. Some of these were greatly swollen. In others the Nissl granules had disappeared, especially in the perinuclear areas, and the nucleus in some was eccentric in position. Neuronophagia of some of these cells could be seen (fig. 4).

Thrombi were also found in the myocardial vessels (fig. 5). Other viscera, such as the liver, kidney and spleen, showed passive hyperemia and rather marked parenchymatous and fatty degeneration (liver) but no distinctive changes. The mass in the neck proved to be a mixed tumor of the parotid gland type.

Specimens of tissue from the brain and lung were examined for nickel, dimethylglyoxime being used for the development of a color reaction.[7] This test is of sufficient sensitivity to demonstrate the presence of 0.001 mg. of nickel. The extract from 3 Gm. of lung gave a strong test, bright red. Three grams of brain tissue gave a much weaker but a very definite pink red. The same method was used in testing 4 Gm. of tissue from the brain in a case of meningitis which gave no color change, the solution remaining colorless by contrast. Blood examined post mortem failed to give a test for carbon monoxide.

SUMMARY

The clinical and pathologic features of this case correspond closely with those of the cases previously reported. The histologic changes in the lung are quite

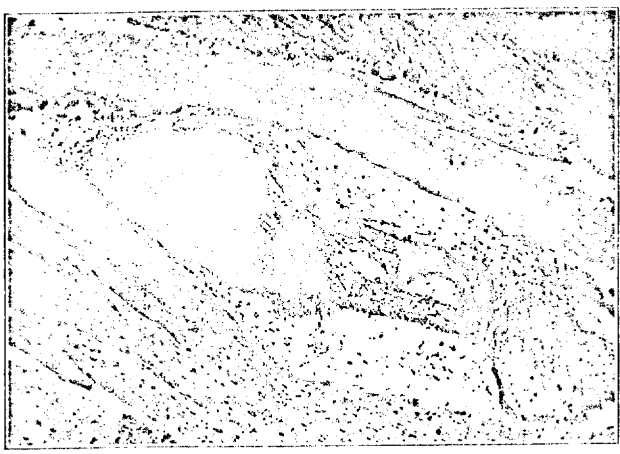

Fig. 5.—Thrombus in a vein in the myocardium. Hematoxylin and eosin stain.

unusual and correspond more nearly with descriptions of observations in the lungs damaged by irritating substances such as phosgene. The changes in the capillaries and arterioles explain the presence of multiple small hemorrhages; also the degenerative changes and areas of anemic necrosis in the brain. That these changes are due to the nickel is indicated by the presence of this metal in relatively large amounts in the tissues analyzed. The lesions were not the result of carbon monoxide present, since at that early stage, seven days, sufficient carbon monoxide should have been present to give a positive test if it were the substance that had caused the widespread lesions. Furthermore, carbon monoxide does not cause the type of lesion in the lung present in this case. Armit [5] demonstrated that the nickel appeared to be transported in the blood in a condition of colloidal solution. The metal is gradually absorbed from the lung in animals poisoned with nickel carbonyl, to such a degree that recovery is possible. The symptoms of extreme dyspnea, nausea and vomiting, restlessness and hyperactivity of reflexes may in part at least have been due to the cerebral lesions.

7. Armit, H. W., and Harden, A.: Quantitative Estimation of Small Quantities of Nickel in Organic Substances, Proc. Roy. Soc. London 77: 420, 1906.

OSTEITIS TUBERCULOSA
CYSTICA OF FIBI
AND TIBIA

SAMUEL SANES, M
AND
WARREN S. SMITH,
BUFFALO

Following its introduction by Jüng
term "osteitis tuberculosa multiplex c
to cystlike degeneration of the b
involvement, characterized by the
mild pain and swelling of the affecte
roentgenographic observations and
course. Not infrequently associated
and Boeck's sarcoid. Histologically,
epithelioid and giant cell tubercles,
caseation. Tubercle bacilli were ra
in examined tissue; guinea-pig inocul
gave positive results for tuberculosis.

In the past year Van Alstyne and C
the literature on osteitis tuberculosa
stated that in all the reported cases
restricted to the small bones of th
Theirs was the first authentic case
lesions in the long bones. They prese
aged 32, who exhibited destructive c
ulna, the left radius, and both humer
etiology was proved by histologic a
methods, and by guinea-pig inoculat
its rarity, verified histopathology an
port, we record an additional case of o
multiplex cystica of the long bones.

REPORT OF CASE

E. S., a white American woman, marrie
in the service of Dr. W. W. Plummer, Sept.
of pain and swelling of both lower leg
duration. At the age of 30 the patient had
father and daughter had died of pulmonar
brother was suffering from the same disea

During July, 1932, the patient had note
in both lower legs. In a short time the sy
leg subsided appreciably. The pain in the
persisted as a dull ache, which was intensifi
was walking or when the leg was bump
history of trauma or loss of weight. Th
The inguinal lymph nodes were not enlarg
and tenderness were found over the lowe
fibula. The right ankle showed no pain or li

The left leg was not remarkable. The
between 98 and 99 F.; the pulse was 80
rate was 20. Laboratory examination sh
90 per cent; leukocytes, 9,700 per cubic
normal. The Kahn test was negative. Ro
(Dr. E. C. Koenig) of the lower legs and
right fibula, about one-half inch above the to
decreased density of the bone tissue with a
excavation, which was cystlike in appeara
thickening of the periosteum on the outer si
excavation. Otherwise, the bone tissue of
fibula was well within average limits. T
foot and ankle did not show any evidence of
parable to that in the right fibula. Exami
indicated increased density of lung markin
right interscapular region, with small calca

At operation (Dr. F. N. Potts), Septem
of destruction was found in the lower end

From the Pathological Laboratory and Orthopedi
General Hospital, and University of Buffalo Schoo

1. Jüngling, Otto: Fortschr. a. d. Geb. d. Rön
1920.
2. Van Alstyne, G. S., and Gowen, G. H.: J.
15: 193 (Jan.) 1933.

Bull. Soc. Chim. Biol. 19:342-352, 1937

Biliary Excretion of Nickel

by M. F. Caujolle

(Presented at the Meeting of December 15, 1936)

After the intravenous injection of nickel chloride in dogs, the nickel consistently passed into the bile but the level of biliary elimination of this metal was minimal at least in the first hours following administration.

After having established the modalities of biliary elimination of cobalt (8), it seemed interesting to us to research to what degree nickel may follow the same route of excretion. The observations published up to now on the biliary elimination of nickel are not very numerous. In 1858 Kletzinsky (14) pointed out for the first time the possibility that nickel was eliminated by the bile. In 1860, Wichert (18), having made a dog with a biliary fistula ingest a gram of nickel sulfate dissolved in some milk, found nickel in bile secreted for 12 hours following the ingestion. Lehmann (15) published in 1909, the results of numerous experiments done on cats and dogs; after administration per os of variable quantities of nickel chloride, sulfate or acetate (up to 12 mg metallic Ni per kg), nickel was generally revealed in the bile and sometimes the concentration of nickel was found to be even higher in the bile than in the blood. On the other hand, Mascherpa in 1927, reported never having found nickel in the bile of guinea pigs having received per os up to 500 mg of metallic nickel (16). We, ourselves, in 1929 after the intravenous injection of 50 mg of nickel chloride to a 21.3 kg chloralosed dog were able to reveal nickel in the bile secreted during the second hour following injection (6). We have not been able to find other data on the significance of the biliary elimination of nickel. The aim of this present work was to determine the immediate value of the hepatobiliary emunctory in regard to nickel in the dog with an extemporaneous choledochal fistula, under anesthesia of chloralose alone or chloralose with morphine or "pantopon" with Roche's sommifacient.

Techniques Utilized

Physiological Technique-To create the choledochal fistulas, the physiologic technique followed is identical to that already described in our work on the biliary elimination of cobalt (8); all the samples of bile collected were strictly lacking in blood.

The injections of nickel chloride dissolved in warm physiologic serum were always done by the intravenous route in the central tip of the saphena. The injected solutions of $NiCl_2$ were generally very diluted.

Chemical Technique--We were very closely inspired by the works of G. Bertrand and his student on the nickel element in arable land (3), plants (2) and animals (1); having adopted the method created and tested by these authors to which we referred (4), we will limit ourselves to indicating the detail of the method of operation followed to isolate, characterize, and measure nickel in samples of bile collected by the fistulas.

The bile was collected in rubber bags attached by a ligature to the end of the canula, each sample was poured into porcelain capsules, the bags were rinsed three times with 3 to 4 cm^3 of distilled water, the rinsing water was mixed with the bile and the contents of the capsules were evaporated in an incubator at $105-110^{\circ}$. The dry extract was burned in muffle furnace without surpassing dark red, this calcination was sometimes quite long. The ashes were methodically drained into hydrochloric acid; the solution obtained contained besides nickel, all the elements soluble in a hydrochloric milieu and in particular, iron, which is normally present in appreciable quantities in bile (13).

This hydrochloric solution was evaporated to dryness in a water-bath, the residue is redissolved in a few cm^3 of boiling distilled water and the aqueous solution thus obtained after cooling was treated with double its volume of officinal ammonia; it was filtered, the precipitate was washed and filtered three times with ammonia. The filtrant and washing liquid were combined which contained nickel in the form of the complex Ni $(NH_3)_6^{++}$, in a 500 cm^3 Erlenmeyer; a few cm^3 of lime water was added and it was brought progressively to boiling: large quantitites of ammonia were released;

boiling was maintained up until the point of total elimination of the ammonia by adding as needed more lime water so that at the end of the operation the presence of a small quantity of free lime assured a milieu that was strictly alkaline to phthalein. During this lime water treatment, a whitish precipitate was formed, more or less abundant, which included all the nickel contained in the initial hydrochloric solution (4); it was separated by filtration.

The precipitate was dissolved in (q.s.) officinal 10% hydrochloric acid; after dissolution, ammonium acetate was added so as to change the milieu to acetic acidity, then a prolonged stream of pure hydrogen sulfide (filtered by a cotton tampon); the nickel was precipitated to the form of nickel sulfide; this nickel sulfide was not pure--it was mixed with other metallic sulfides such as CuS and COS, but in quantities virtually negligeable for the purpose of our work.

The nickel sulfide collected on a filter without charcoal was washed with 5% solution of acetic acid saturated with hydrogen sulfide; the precipitate and the filter, dried in an incubator at 105° were calcined in a capsule for about 20 minutes in a muffle furnace, the residue composed of nickel oxide was dissolved in a few cm of officinal half-strength hydrochloric acid; the hydrochloric liquid and the rinsing waters of the capsule were combined in a beaker (?).

It was brought to boiling and a large amount of an alcoholic solution of 2% dimethyl glyoxime added, then it was removed from the heat and ammonia added until a slightly alkaline reaction was obtained. The bright red crystalline precipitate of the dimethylglyoximate complex of nickel-dimethylglyoxime was collected in a tared Gooch crucible; it was washed with warm water, dried at 100° and weighed.

If the amount of the precipitate is insufficient to allow a weight determination, the measurement can be done by redissolving the well-dried dimethylgloxime complex in boiling chloroform; then this chloroform solution is slowly made to dissolve in a capsule of as little depth as possible; the red residue thus obtained is compared to the controls prepared under the same conditions from nickel chloride solutions of known concentration (containing 10^{-1} and 10^{2} milligrams of nickel).

The weighing method enables determination of one-half milligram; colorimetric approximation gives the possibility of evaluating quantities on the order of a hundredth of a milligram.

Experimental Results

The toxicity of nickel chloride for dogs is quite poorly known, the lethal dose per kilogram of animal after administration by the intravenous route not being fixed (1): Gmelin (11) indicated that the intravenous injection of 600 mg of nickel chloride was immediately fatal for a dog and a dose of 300 mg was very toxic and quite rapidly caused death. In the absence of precise evidence, we used amounts of nickel chloride essentially similar to those of cobalt chloride used in the study of the biliary elimination of that metal. Actually, from the first three attempts, it appeared to us that the nickel chloride could be considered slightly more toxic than the cobalt chloride, at least for dogs and under the conditions of administration adopted; we were then led to diminish the injected doses, to divide them up and to give the injections only with extreme slowness (7).

Dog 1--♂, 18 kg. Anesthesia at 8:30 with 5 cm^3 of solution of 2% morphine chlorhydrate subcutaneously, followed at 8:55 by 1.80 g chloralose dissolved in 100 cm^3 of warm physiologic serum intravenously. Two injections of nickel chloride solution of 128.8 mg metallic Ni at 10:33-10:35 and 257.6 mg metallic Ni at 12-12:04. The subject received 20.29 mg of metallic Ni per kilogram: he died at 12:05. From 10:30 to 12:04, he secreted 2 cm^3 of bile, containing no nickel. The gall bladder sampled at autopsy contained no nickel.

Dog 2--♂, 25 kg. Anesthesia at 9:30 with 2.05 g chloralose dissolved in physiologic serum and administered in two consecutive injections. Two injections of $NiCl_2$ solution: 160.95 mg of Ni-metal at 10:18 to 10:20, and 261 mg of Ni-metal at 12:13-12:19. Death occurred shortly after the second injection, the subject having received less than 17 mg Ni-metal per kilogram. From the first injection, the abundant biliary secretion was slowed down; from 10:20 until the time of death, less than 1 cm^3 of bile was collected, containing minute traces of nickel: the bladder and the gall bladder taken at autopsy contained 1.00 mg nickel or 2.27 mg Ni-metal per 100 g of organ.

Dog 3--♀, 15 kg. Anesthesia at 8:55 with 1.50 g of chloralose dissolved in 80 cm^3 of physiologic serum received at 14:40 a second injection of 1 g chloralose dissolved in 20 cm^3 of physiologic serum. The subject secreted little bile: he died at 16 h. after having received in 6 injections, 29.9 mg Ni-metal/kg; the results are shown in Table I.

Table I

1 Horaire des injections et des prélèvements.	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm^3	4 Quantités correspondantes de Ni en mg.
9 h. 55 — 9 h. 57	43,5	—	—
10 h. 5 — 10 h. 6	43,5	—	—
9 h. 55 — 11 h.	—	0.6	traces légères 5
12 h. 15 — 12 h. 17	72,5	—	—
12 h. 25 — 12 h. 29	58,0	—	—
15 h. 37 — 15 h. 43	72,5	—	—
15 h. 33 — 15 h. 56	54,0	—	—
11 h. — 16 h.	—	0,5	néant 6

1--Schedule of injections and samplings
2--Ni injected in mg
3--Volume of bile collected in cm^3
4--Corresponding quantities of Ni in mg
5--Slight traces
6--None

Dog 4--♂, 17 kg. Anesthesia at 9 h. with 2 cm^3 of 2% morphine chlorhydrate solution subcutaneously, followed at 9:23 by 1.80 g chloralose dissolved in 100 cm^3 of warm physiologic solution intravenously. After having received 7.45 mg of Ni-metal/kg, the animal was electrocuted at 18:25; the results are shown in Table II. Biliary secretion stopped at 16:30.

The five samples of bile collected contained less than 0.20 mg of metallic nickel.

Dog 5--♂, 9 kg. Anesthesia at 8:20 with 5 cm^3 of 2% morphine chlorhydrate solution subcutaneously, followed at 8:30 by 0.90 g of chloralose dissolved in 80 cm^3 of warm physiogic serum intravenously. From 9:47 to 10:02, a solution of nickel chloride containing 87.37 mg nickel-metal or 9.7 mg/kg was injected. The subject died at 18 h. having only secreted 4.2 cm^3 of bile; this bile contained only slight traces of nickel.

Table II

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm^3	4 Quantités correspondantes de Ni en mg.
10 h. 35 — 10 h. 39	43,5	—	—
10 h. 39 — 11 h. 5	—	1	traces légères 5
11 h. 5 — 12 h.	—	2	traces légères 5
12 h. — 12 h. 2	43,5	—	—
12 h. — 14 h.	—	5	traces légères 5
14 h. — 14 h. 2	43,5	—	—
14 h. — 15 h. 30	—	8	traces
15 h. 30 — 16 h. 50	—	4	traces
Les cinq échantillons de bile réunis contiennent moins de 0,20 mg. de nickel métal 6			

1--Schedule of injections and samplings
2--Ni injected in mg
3--Volume of bile collected in cm^3
4--Corresponding quantities of Ni in mg
5--Slight traces
6--The five samples of bile together contained less than 0.20 mg of Ni-metal

Dog 6--♂, 15 kg. Anesthesia at 9 h. with 1.50 g of chloralose dissolved in 60 cm^3 of warm physiologic serum intravenously, followed at 14 h. by a second injection of 0.50 g dissolved in 30 cm^3 of warm physiologic serum. From 10:02 to 10:45, we injected into the saphena a solution of nickel chloride containing 150 mg of metallic Ni or 10 mg/kg. The subject was electrocuted at 17:50. In the bile collected from 10:02-11, there was no nickel; from 11 h., nickel appeared in the bile in very small quantities; from 11:30 to 17:50, all the samples of bile collected also contained nickel but also in small quantities. The total elimination was on the order of 0.15 mg for a total biliary volume of about 20 cm^3.

Dog 7--o, 23 kg. Anesthesia was given by 3 intravenous injections of chloralose dissolved in warm physiologic serum: 2.30 g at 9:02,--0.20 g at 9:10,--1.50 g at 12:45. The biliary section slowed down up to 17:30.

The fourth injection of $NiCl_2$ was done as an exception in a mesenteric vein exposed by laparatomy; the purpose was to find whether the hepatic permeability would be greater when the nickel left the hepatic lobe by the small channels of the portal system; the experiment being very shocking for the animal already traumatized by the creation of the choledochal fistula, it could not be done again.

The subject who had received 24.77 mg Ni/kg was electrocuted at 19:45; the results are collected in Table III.

Table III

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
10 h. 16 — 10 h. 20	100,05	—	—
10 h. 16 — 12 h.	—	1,1	traces légères 5
12 h. — 14 h.	—	1,9	traces légères
12 h. 6 — 12 h. 16	130,60	—	—
14 h. — 15 h.	—	2,7	traces légères
15 h. — 16 h. 10	—	2,5	traces
15 h. 6 — 15 h. 19	174,00	—	—
16 h. 19 — 17 h. 30	—	6,9	traces
17 h. 30 — 17 h. 50	—	2	traces légères
17 h. 50 — 18 h. 35	—	—	traces légères
18 h. 35 — 18 h. 43	165,30	0,2	—
18 h. 35 — 18 h. 45	—	0	0

Les sept échantillons de bile réunis contiennent un peu moins d'un mg. de nickel 6

1--Schedule of injections and samples
2--Ni injected in mg
3--Volume of bile collected in cm^3
4--Corresponding quantities of Ni in mg
5--Slight traces
6--The seven samples of bile together contained less than a mg of nickel.

Dog 8-- ♂, 18 kg. The anesthesia was assured by two intravenous injections of chloralose dissolved in warm physiologic serum: 2 g at 8:25 and 1,50 g at 14 h. The biliary secretion was abundant and was maintained that way for the duration of the experiment. The subject having 25.3 mg of metallic nickel was electrocuted at 19 hours; Table IV summarizes the results obtained.

Table IV

1 Horaire des injections et des prélèvements	2 Ni injecté en mg	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg
11 h. — 11 h. 5	65,0	—	—
11 h. — 12 h.	—	2,5	néant 5
12 h. — 12 h. 10	130,0	—	—
12 h. — 15 h.		5,4	0,12
15 h. — 15 h. 10	130,0	—	—
15 h. — 18 h.			0,59
18 h. — 18 h. 10	130,0	—	—
18 h. — 19 h.	-	1,5	0,18

1--Schedule of injections and samples
2--Ni injected in mg
3--Volume of bile collected in cm^3
4--Corresponding quantities of Ni in mg
5--None

Dog 9--♀, 16.5 kg. Anesthesia at 14:15 with 5 cm^3 of 2% solution of morphine chlorhydrate subcutaneously, followed at 14:50 by 4 cm^3 of Roche's injectable somnifacient. Biliary secretion was scarce. The subject who had received a total 27.5 mg of metallic nickel was electrocuted at 22:40; Table V summarizes the results obtained.

Dog 10--♂, 19 kg. Anesthesia at 8:45 with 2 cm^3 of 2% morphine chlorhydrate subcutaneously, followed at 9 h by 4 cm^3 of Roche's injectable somnifacient. Four successive intravenous injections of $NiCl_2$ were given, in one injection representing 121.8 mg of Ni-metal at 10-10:02 and three injections each representing 243.6 mg at 12-12:02, 14:45-14:47 and 16:30-16:32; the subject received a total of 44.9 mg Ni-metal/kg. The subject, deeply poisoned, secreted from 10 to 18:20 barely 3 cm^3 of bile, containing less than 0.10 mg nickel. He was sacrificed by electrocution at 18:20.

Table V

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm^3	4 Quantités correspondantes de Ni en mg.
16 h. – 16 h. 5	65,0	—	
16 h. — 17 h.	—	2,0	trace très légère 5
17 h. — 17 h. 10	130,0	—	—
17 h. — 19 h.	—	3,9	0,17
19 h. — 19 h. 10	130,0	-	—
19 h. — 21 h.	—	2,9	0,19
21 h. — 21 h. 10	130.0	—	—
21 h. — 22 h. 40	—	2,7	0,10

1--Schedule of injections and samplings
2--Ni injected in mg
3--Volume of bile collected in cm^3
4--Corresponding quantities of Ni in mg

We collected in Table VI the total of the results obtained. These results valid for the conditions of the technique and the duration of our experiments show that the biliary elimination of nickel took place in a nearly constant manner without ever attaining a notable quantitative significance. This elimination was early; often it was manifested in the hour following the time of the first injection; it seemed to be continued beyond the duration of our experiments, yet the diminution of biliary secretion consistently observed restricted it.

These results compared with those already published on cobalt show a clear and remarkable difference between the two metals, Ni and Co, however their chemistry is very similar. If chloralose seems to favor the biliary elimination of nickel as it favors the elimination of cobalt, it nevertheless remains that the biliary elimination of nickel always remains very much less significant than the biliary elimination of cobalt. These facts are

Table VI

N° d'ordre	1 Poids en kg.	2 Anesthésiques utilisés	3 Quantités de nickel utilisées en mg. totale	4 par kilog	5 Durée réelle de l'expérience	6 Volume de bile recueilli en cm³	7 Quantité totale de Ni présente dans la bile recueillie en mg.
1	18	morphine + chloralose	128,80	20,5	1 h. 28 (†)	2,0	néant 8
2	25	chloralose	422,00	17,0	2 h. (†)	(*)	traces très légères 9
3	15	chloralose	354,00	22,9	1 h. 20 (†)	1,1	1,06
4	17,5	morphine + chloralose	130,50	7,45	7 h. 50	21,0	0,20
5	9	morphine + chloralose	87,37	9,7	8 h. 13	4,2	traces très légères
6	15	chloralose	150,00	10,0	7 h. 48	env. 20	0,55
7	23	chloralose	569,25	24,8	9 h. 29	20,3	< 1,00
8	18	chloralose	455,00	25,3	8 h.	10,1	0,89
9	16,5	morphine + somnifène	455,00	27,5	6 h. 40	11,5	0,16
10	19	morphine + somnifène	852,60	44,9	8 h. 20	3,0	< 0,10

* Le dosage a été fait sur 1 cm³ de bile obtenu par la fistule, et sur la vésicule biliaire et son contenu, prélevés à l'autopsie. 10

1--Weight in kg
2--Anesthetics used
3--Quantities of nickel used in mg
4--Per kilogram
5--Actual duration of the experiment
6--Volume of bile collected in cm^3
7--Total quantity of Ni present in the collected bile in mg
8--None
9--Very slight traces
10--Determination was done in 1 cm^3 of bile obtained by the fistula, and on the gall bladder and its contents, sampled at autopsy.

perhaps dependent on the rapidity with which nickel injures the hepatic cell; the first results of an anatomic-pathologic study, performed in collaboration with M.R. Argaud, show that nickel is actually more aggressive towards the tissues than cobalt (5, 7).

Conclusions

The totality of the results reported establishes that quantities of nickel being eliminated by the bile under the conditions of our experiments always remain minimal; if these quantities seemed to be slightly increased under the choleretic effect of chloralose, they remained in every case less than those which were observed in the study of biliary elimination of cobalt done under analogous experimental conditions.

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19:342-352 (1937)

L'élimination biliaire du nickel,

par M. F. CAUJOLLE.

(*Mémoire présenté à la séance du 15 décembre 1936*).

Après injection intraveineuse de chlorure de nickel on observe chez le Chien le passage du nickel dans la bile d'une manière constante, mais le taux de l'élimination biliaire de ce métal est minime, tout au moins dans les premières heures consécutives à l'administration.

Après avoir établi les modalités de l'élimination biliaire du cobalt [8], il nous a paru intéressant de rechercher dans quelle mesure le nickel pouvait suivre la même voie d'excrétion. Les observations jusqu'ici publiées sur l'élimination biliaire du nickel sont peu nombreuses. En 1858, KLETZINSKY [14] signale pour la première fois la possibilité pour le nickel de s'éliminer par la bile. En 1860, WICHERT [18] ayant fait ingérer à un Chien porteur d'une fistule biliaire un gramme de sulfate de nickel dissous dans du lait, put mettre en évidence le nickel dans la bile secrétée dans les douze heures consécutives à l'ingestion. LEHMANN [15], publie, en 1909, les résultats de nombreuses expériences pratiquées sur le Chat et le Chien ; après administration *per os* de quantités variables de chlorure, sulfate ou acétate de nickel (jusqu'à 12 mg. de Ni métal par kilog), le nickel a été généralement décelé dans la bile et parfois même la concentration du nickel se trouva être plus élevée dans la bile que dans le sang. Par contre, MASCHERPA, en 1927, rapporte n'avoir jamais trouvé de nickel dans la bile de Cobayes ayant reçu *per os* jusqu'à 500 mg. de nickel-métal [16]. Nous-même, en 1929, après injection intraveineuse de 50 mg. de chlorure de nickel à un Chien de 21 kg.,3 chloralosé, nous avons pu déceler le nickel dans la bile secrétée pendant la deuxième heure consécutive à l'injection [6]. Nous n'avons pu relever d'autres données sur l'importance de l'élimination biliaire du nickel. Le but du présent travail a été de déterminer la valeur immédiate de l'émonctoire hépato-biliaire vis-à-vis du nickel chez le Chien porteur d'une fistule cholédocienne extemporanée, sous anesthésie au chloralose seul, au chloralose associé à la morphine, ou au pantopon associé au somnifène Roche.

Techniques utilisées.

TECHNIQUE PHYSIOLOGIQUE. — Pour établir les fistules cholédociennes la technique physiologique suivie est identique à celle déjà décrite dans notre travail sur l'élimination biliaire du cobalt [8] ; tous les échantillons de bile recueillis étaient rigoureusement exempts de sang.

Les injections de chlorure de nickel, dissous dans du sérum physiologique tiède, ont toujours été pratiquées par voie intraveineuse, dans le bout central de la saphène. Les solutions injectées de Cl_2 Ni étaient généralement très diluées.

TECHNIQUE CHIMIQUE. — Nous nous sommes très étroitement inspiré des travaux de M. G. BERTRAND et de ses élèves sur le nickel constant de la terre arable [3], des végétaux [2] et des animaux [1] ; ayant adopté la méthode créée et éprouvée par ces auteurs, auxquels nous renvoyons [4], nous nous limiterons à indiquer le détail du mode opératoire suivi pour isoler, caractériser et doser le nickel dans les échantillons de bile récoltés par les fistules.

La bile est recueillie dans des sachets de caoutchouc fixés par une ligature au bord de la canule, chaque échantillon est décanté dans des capsules de porcelaine, les sachets sont rincés trois fois avec 3 à 4 cm³ d'eau distillée, les eaux de rinçage sont réunies avec la bile et le contenu des capsules est évaporé à l'étuve à 105°-110°. On calcine l'extrait sec au four à moufle sans dépasser le rouge sombre, cette calcination est parfois assez longue. Les cendres sont épuisées méthodiquement à l'acide chlorhydrique ; la solution obtenue contient à côté du nickel tous les éléments solubles en milieu chlorhydrique et en particulier du fer, qui est normalement présent en quantités appréciables dans la bile [13].

On évapore à siccité au bain-marie cette solution chlorhydrique, on redissout le résidu dans quelques cm³ d'eau distillée bouillante et on traite la solution aqueuse ainsi obtenue, après refroidissement, par le double de son volume d'ammoniaque officinale ; on filtre, on lave le précipité et le filtre trois fois à l'ammoniaque. On réunit le filtrat et les liqueurs de lavages, qui contiennent le nickel à l'état de complexe Ni $(NH_3)_6^{++}$, dans un erlenmeyer de 500 cm³ ; on ajoute quelques cm³ d'eau de chaux et l'on porte progressivement à l'ébullition : de grandes quantités d'ammoniac se dégagent ; on maintient l'ébullition jusqu'à élimination totale de l'ammoniac, en ajoutant au besoin de nouvelles quantités d'eau de chaux de manière à ce que jusqu'en fin d'opération la présence d'une petite quantité de chaux libre assure au milieu une réaction franchement alcaline à la phtaléine. Pendant ce traitement à l'eau de chaux se forme un précipité blanchâtre plus ou moins abondant qui renferme tout le nickel contenu dans la solution chlorhydrique initiale [4], on le sépare par filtration.

Le précipité est dissous dans q. s. d'acide chlorhydrique officinal à 10 p. 100, après dissolution on ajoute de l'acétate d'ammonium de manière à passer en milieu d'acidité acétique, ensuite par un courant prolongé d'hydrogène sulfuré pur (filtré sur un tampon de ouate), on précipite le nickel à l'état de sulfure de nickel ; ce sulfure de nickel n'est pas pur, il est mélangé à d'autres sulfures métalliques, tels que SCu et SCo, mais en quantité tout à fait négligeables pour l'objet de notre travail.

Le sulfure de nickel recueilli sur un filtre sans cendres est lavé avec une solution à 5 p. 100 d'acide acétique saturée d'hydrogène sulfuré ; le précipité et le filtre, séchés à l'étuve à 105°, sont calcinés dans une capsule pendant 20 minutes environ au four à moufle, le résidu constitué par de l'oxyde de nickel est dissous dans quelques cm³ d'acide chlorhydrique officinal au demi ; on réunit la liqueur chlorhydrique et les eaux de rinçage de la capsule dans un bécher.

On porte à l'ébullition et on ajoute un grand excès de solution alcoolique à 2 p. 100 de diméthylglyoxime, puis on retire du feu et l'on ajoute de l'ammoniaque jusqu'à réaction légèrement alcaline. Le précipité cristallin, rouge vif, du complexe diméthylglyoximate de nickel-diméthylglyoxime est recueilli sur un creuset de Gooch taré ; on le lave à l'eau chaude, on sèche à 100° et on pèse.

Si l'importance du précipité est insuffisante pour permettre un dosage pondéral, on achève la détermination en redissolvant le complexe diméthylgloximique bien desséché dans du chloroforme bouillant, puis on fait évaporer doucement cette solution chloroformique dans une capsule de porcelaine à fond aussi plan que possible ; on compare le résidu rouge ainsi obtenu avec des témoins préparés dans les mêmes conditions à partir de solutions de chlorure de nickel de titre connu (contenant 10^{-1} et 10^{-2} milligramme de nickel).

La méthode pondérale permet de doser un demi-milligramme de nickel ; l'approximation colorimétrique nous a donné la possibilité d'évaluer des quantités de l'ordre du centième de milligramme.

Résultats expérimentaux.

La toxicité du chlorure de nickel pour le Chien est assez mal connue, la dose mortelle par kilog d'animal après administration par voie intraveineuse n'est pas fixée [1] ; GMELIN [11] indique que l'injection intraveineuse de 600 mg. de chlorure de nickel est immédiatement mortelle pour un Chien, que la dose de 300 mg. est très toxique et détermine assez rapidement la mort. En l'absence d'une documentation précise, nous avons mis en œuvre des doses de chlorure de nickel sensiblement voisines de celles de chlorure de cobalt utilisées lors de l'étude de l'élimination biliaire de ce métal. En réalité, dès les trois premiers essais, il nous est apparu que le chlorure de nickel pouvait être considéré comme légèrement plus toxique que le chlorure de cobalt, tout au moins pour le Chien et dans les conditions d'administration adoptées ; nous avons donc été conduits à diminuer les doses injectées, à les fragmenter et à ne pousser les injections qu'avec une extrême lenteur [7].

Chien 1. — ♂, 18 kg. Anesthésié à 8 h. 30 avec 5 cm³ de solution à 2 p. 100 de chlorhydrate de morphine par voie sous-cutanée, suivis à 8 h. 55 de 1 g.,80 de chloralose dissous dans 100 cm³ de sérum physiologique tiède par voie intraveineuse. Deux injections de solution de chlorure de nickel, soit 128 mg.,8 de Ni-métal à 10 h. 33-10 h. 35 et 257 mg.,6 de Ni-métal à 12 h.-12 h. 4. Le sujet a reçu 20 mg.,29 de Ni-métal par kilog : il succombe à 12 h. 5. De 10 h. 30 à 12 h. 4, il a secrété 2 cm³ de bile, ne contenant pas de nickel. La bile vésiculaire prélevée à l'autopsie ne contient pas de nickel.

Chien 2. — ♂, 25 kg. Anesthésié à 9 h. 30 avec 2 g.,05 de chloralose dissous dans du sérum physiologique et administré en deux injections consécutives. Deux injections de solution de Cl_2Ni : soit 160 mg.,95 de Ni-métal à 10 h. 18-10 h. 20, et 261 mg. de Ni-métal à 12 h. 13-12 h. 19. La mort survient peu après la seconde injection, le sujet ayant reçu moins de 17 mg. de Ni-métal par kilog. Dès la première injection, la sécrétion biliaire fort abondante s'est ralentie ; de 10 h. 20 jusqu'à l'heure de la mort, on a recueilli moins d'un cm³ de bile, contenant des traces infimes de nickel ; la vésicule et la bile vésiculaire, recueillies à l'autopsie, contiennent 1,00 mg. de nickel, soit 2,27 mg. de Ni-métal par 100 g. de matière.

Chien 3. — ♀, 15 kg. Anesthésiée à 8 h. 55 avec 1 g.,50 de chloralose dissous dans 80 cm³ de sérum physiologique, reçoit à 14 h. 40 une seconde injection de 1 g. de chloralose dissous dans 20 cm³ de sérum physiologique. Le sujet secrète peu de bile ; il succombe à 16 h. après avoir reçu en 6 injections 29,9 mg. de Ni-métal par kilog ; les résultats sont réunis dans le tableau I.

TABLEAU I.

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
9 h. 55 — 9 h. 57	43,5	—	—
10 h. 5 — 10 h. 6	43,5	—	—
9 h. 55 — 11 h.	—	0,6	traces légères 5
12 h. 15 — 12 h. 17	72,5	—	—
12 h. 25 — 12 h. 29	58,0	—	—
15 h. 37 — 15 h. 43	72,5	—	—
15 h. 33 — 15 h. 56	54,0	—	—
11 h. — 16 h.	—	0,5	néant 6

Chien 4. — ♂, 17,5 kg. Anesthésié à 9 h. avec 2 cm³ de solution à 2 p. 100 de chlorhydrate de morphine par voie sous-cutanée, suivis à 9 h. 23 de 1 g.,80 de chloralose dissous dans 100 cm³ de sérum physiologique tiède par voie intraveineuse. Après avoir reçu 7,45 mg de Ni-métal par kilog, l'animal est électrocuté à 18 h. 25 ; les résultats sont réunis dans le tableau II. La sécrétion biliaire se suspend à 16 h. 30.

TABLEAU II.

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
10 h. 35 — 10 h. 39	43,5	—	—
10 h. 39 — 11 h. 5	—	2	traces légeres 5
11 h. 5 — 12 h.	—	2	traces légères 5
12 h. — 12 h. 2	43,5	—	—
12 h. — 14 h.	—	5	traces légères 5
14 h. — 14 h. 2	43,5	—	—
14 h. — 15 h. 30	—	8	traces
15 h. 30 — 16 h. 50	—	4	traces
Les cinq échantillons de bile réunis contiennent moins de 0,20 mg. de nickel-métal 6			

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Les cinq échantillons de bile réunis contiennent moins de 0,20 mg. de nickel-métal.

Chien 5. — ♂, 9 kg. Anesthésié à 8 h. 20 avec 5 cm³ de solution à 2 p. 100 de chlorhydrate de morphine par voie sous-cutanée suivis à 8 h. 30 de 0 g.,90 de chloralose dissous dans 80 cm³ de sérum physiologique tiède par voie intraveineuse. De 9 h. 47 à 10 h. 2, on injecte une solution de chlorure de nickel contenant 87 mg.,37 de nickel métal, soit 9 mg.,7 par kilog. Le sujet succombe à 18 heures n'ayant secrété que 4 cm³,2 de bile ; cette bile ne contient que de faibles traces de nickel.

Chien 6. — ♂, 15 kg. Anesthésié à 9 heures avec 1 g.,50 de chloralose dissous dans 60 cm³ de sérum physiologique tiède par voie intraveineuse, suivis à 14 heures d'une seconde injection de 0 g.,50 dissous dans 30 cm³ de sérum physiologique tiède. De 10 h. 2 à 10 h. 45, on injecte dans la saphène une solution de chlorure de nickel contenant 150 mg. de Ni-métal, soit 10 mg. par kilog. Le sujet est électrocuté à 17 h. 50. Dans la bile récoltée de 10 h. 2 à 11 h., il n'y a pas de nickel ; à partir de 11 heures, le nickel appa-

TABLEAU III.

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
10 h. 16 — 10 h. 20	100,05	—	—
10 h. 16 — 12 h.	—	1,1	traces légères 5
12 h. — 14 h.	—	1,9	traces légères
12 h. 6 — 12 h. 16	130,50	—	—
14 h. — 15 h.	—	2,7	traces légères
15 h. — 16 h. 10	—	7,5	traces
15 h. 6 — 15 h. 19	174,00	—	—
16 h. 10 — 17 h. 30	—	6,9	traces
17 h. 30 — 17 h. 50	—	2	traces légères
17 h. 50 — 18 h. 35	—	—	traces légères
18 h. 35 — 18 h. 43	165,30	0,2	—
18 h. 35 — 18 h. 45	—	0	0

Les sept échantillons de bile réunis contiennent un peu moins d'un mg. de nickel 6

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rait dans la bile mais en très faible quantité ; de 11 h. 30 à 17 h. 50, tous les échantillons de bile récoltés contiennent également du nickel mais toujours en faible quantité. L'élimination totale est de l'ordre de 0,15 mg. pour un volume biliaire total d'environ 20 cm³.

Chien 7. — ♂, 23 kg. L'anesthésie est assurée par 3 injections intraveineuses de chloralose dissous dans du sérum physiologique tiède : 2 g.,30 à 9 h. 2, — 0 g.,20 à 9 h. 10, — 1 g.,50 à 12 h. 45. La sécrétion biliaire ralentie jusqu'à 14 heures, devient ensuite abondante jusqu'à 17 h. 30.

La quatrième injection de Cl_2 Ni est pratiquée, par exception, dans une veine mésaraïque mise à nu par laparotomie ; le but poursuivi était de savoir si la perméabilité hépatique serait plus grande lorsque le nickel aborderait le lobule hépatique par les canalicules du système porte ; l'expérience très shockante pour l'animal, déjà traumatisé par l'établissement de la fistule cholédocienne, n'a pu être renouvelée .

Le sujet, qui a reçu 24 mg.,77 de Ni par kilog, est électrocuté à 19 h. 45 ; les résultats sont réunis dans le tableau III.

Chien 8. — ♂, 18 kg. L'anesthésie est assurée par 2 injections intraveineuses de chloralose dissous dans du sérum physiologique tiède : 2 g., à 8 h. 25 et 1 g.,50 à 14 heures. La sécrétion biliaire est abondante et se maintient pendant toute la durée de l'expérience. Le sujet qui a reçu par kilog 25,3 mg. de nickel-métal est électrocuté à 19 heures ; le tableau IV résume les résultats obtenus.

TABLEAU IV.

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
11 h. — 11 h. 5	65,0	—	—
11 h. — 12 h.	—	2,5	néant 5
12 h. — 12 h. 10	130,0	—	—
12 h. — 15 h.	—	5,1	0,12
15 h. — 15 h. 10	130,0	—	—
15 h. — 18 h.	—		0,59
18 h. — 18 h. 10	130,0	—	—
18 h. — 19 h.	—	1,5	0,18

Chien 9. — ♀, 16,5 kg. Anesthésié à 14 h.15 avec 5 cm³ de solution à 2 p. 100 de chlorhydrate de morphine par voie sous-cutanée, suivis à 14 h. 50 de 1 cm³ de somnifène Roche injectable. La sécrétion biliaire est peu abondante. Le sujet, qui a reçu au total 27,5 mg. de nickel-métal, est électrocuté à 22 h. 40 ; le tableau V résume les résultats obtenus.

TABLEAU V.

1 Horaire des injections et des prélèvements	2 Ni injecté en mg.	3 Volumes de bile recueillis en cm³	4 Quantités correspondantes de Ni en mg.
16 h. — 16 h. 5	65,0	—	—
16 h. — 17 h.	—	2,0	trace très légère 5
17 h. — 17 h. 10	130,0	—	—
17 h. — 19 h.	—	3,9	0,17
19 h. — 19 h. 10	130,0	—	—
19 h. — 21 h.	—	2,9	0,19
21 h. — 21 h. 10	130,0	—	—
21 h. — 22 h. 40	—	2,7	0,10

Chien 10. — ♂, 19 kg. Anesthésié à 8 h. 45 avec 2 cm³ de solution à 2 p. 100 de chlorhydrate de morphine par voie sous-cutanée, suivis à 9 heures de 4 cm³ de somnifène Roche injectable. On pratique quatre injections intraveineuses successives de Cl_2Ni, soit une injection représentant 121,8 mg. de Ni-métal à 10 h.-10 h. 2 et trois injections représentant chacune 243,6 mg. à 12 h.-12 h. 2, 14 h. 45-14 h. 47 et 16 h. 30-16 h. 32 ; le sujet reçoit au total 44,9 mg. de Ni-métal par kilog. Le sujet profondément intoxiqué secrète de 10 h. à 18 h. 20 à peine 3 cm³ de bile, contenant moins de 0 mg.,10 de nickel. Il est sacrifié par électrocution à 18 h. 20.

Nous avons réuni dans le tableau VI l'ensemble des résultats obtenus. Ces résultats, valables pour les conditions de technique et de durée de nos expériences, montrent que l'élimination biliaire du nickel se réalise d'une façon à peu près constante sans jamais atteindre une importance quantitative notable. Cette élimination est précoce : elle se manifeste souvent dans l'heure qui suit le moment de la première injection ; elle paraît devoir se

TABLEAU VI.

N° d'ordre	1 Poids en kg.	2 Anesthésiques utilisés	3 Quantités de nickel utilisées en mg.		5 Durée réelle de l'expérience	6 Volume de bile recueilli en cm³	7 Quantité totale de Ni présente dans la bile recueillis en mg.
			totale	4 par kilog			
1	18	morphine + chloralose	128,80	20,3	1 h. 38 (†)	2,0	néant 8
2	25	chloralose	422.00	17.0	2 h. (†)	(*)	traces très légères 9
3	15	chloralose	354,00	22,9	1 h. 20 (†)	1,1	1,00
4	17,5	morphine + chloralose	130.50	7.45	7 h. 50	21,0	0,20
5	9	morphine + chloralose	87,37	9,7	8 h. 13	4,2	traces très légères
6	15	chloralose	150,00	10,0	7 h. 48	env. 20	0,15
7	23	chloralose	569,85	24,8	9 h. 29	20,3	< 1,00
8	18	chloralose	455,00	25,3	8 h.	10,1	0,89
9	16,5	morphine + somnifène	455,00	27,5	6 h. 40	11,5	0,46
10	19	morphine + somnifène	852,60	44,9	8 h. 20	3,0	< 0,10

(*) Le dosage a porté sur 1 cm³ de bile, obtenu par la fistule, et sur la vésicule biliaire et son contenu, prélevés à l'autopsie. 10

prolonger au-delà de la durée de nos expériences, encore la diminution de la sécrétion biliaire constamment observée vient-elle la restreindre.

Ces résultats confrontés avec ceux déjà publiés sur le cobalt, font apparaître une différence nette et remarquable entre ces deux métaux, le Ni et le Co, que la chimie rapproche cependant très étroitement. Si le chloralose paraît favoriser l'élimination biliaire du nickel comme il favorise l'élimination du cobalt, il n'en reste pas moins que l'élimination biliaire du nickel demeure toujours très inférieure en importance à l'élimination biliaire du cobalt. Ces faits sont peut-être sous la dépendance de la rapidité avec laquelle le nickel lèse la cellule hépatique ; les premiers résultats d'une étude anatomo-pathologique, effectuée en collaboration avec M. R. Argaud, montrent en effet que le nickel est bien plus agressif pour les tissus que le cobalt [5, 7].

Conclusions.

L'ensemble des résultats rapportés établit que les quantités de nickel s'éliminant par la bile, dans les conditions de nos expériences, demeurent toujours minimes ; si ces quantités paraissent s'augmenter légèrement sous l'effet cholérétique du chloralose, elles demeurent, en tous cas, inférieures à celles qu'il est permis d'observer dans l'étude de l'élimination biliaire du cobalt effectuée sous des conditions expérimentales analogues.

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GIG. SANIT. 31(8):109. 1966

TOXIC ACTION OF NICKEL WITH PERORAL ADMINISTRATION

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Nickel as a trace element is a constituent of living organisms (A. I. Voynar). It is essential in certain low quantities as a catalyst of several oxidative processes, but if present in a large amount, it may be toxic. For example, plants growing on soil in regions abounding in nickel are known to assume abnormal shapes, while animals in these regions accumulate nickel in their fur, skin, and horns. Sheep and cows, especially young stock, frequently suffer from such eye diseases as keratitis and kerato-conjunctivitis (S. N. Cherkinskiy; A. I. Voynar). Although there are indications that nickel can be toxic regardless of the route of entry into the body, the question of whether it is injurious when ingested is still moot. Moreover, some data seem to suggest that nickel is completely harmless with this route of entry.

The problem of nickel toxicity became particularly acute after nickel and nickel-plated pots came into widespread use because of the possibility that a large amount of the element might be taken up by food during cooking (A. V. Reyser). However, most investigators point out that nickel consumed with food is relatively harmless because a person eating food cooked in a nickel pot will not receive more than 2 mg of the element per kg of body weight a day. And at a dose of

100 μg/kg it does not appear to have a perceptibly toxic effect on experimental animals. Leman shares this view unequivocally.

The conflict in the data presented above prompted us to make a further study of the toxicity of nickel when administered perorally. For this purpose we introduced into the stomachs of white rats through a tube a nickel chloride solution at doses of 0.06, 0.12, and 0.3 mg/kg (in metallic nickel equivalent) once a day for 13 weeks. Each of the 3 experimental groups consisted of 10 animals. The control also consisted of 10 rats given distilled water.

The animals' general condition and behavior were monitored throughout the experiment and they were regularly weighed. The number of erythrocytes and leukocytes in peripheral blood was determined before the experiment, 30 and 60 days from the start, and at the end. The color index and blood catalase activity was studied before the experiment and at the end.

The animals looked completely healthy, remained active, and ate the food offered throughout the experiment. However, in the rats that received 0.12 and 0.3 mg/kg of nickel, the weight increase expressed as a percentage of the baseline value for each group was less at the end of the experiment. The lag was most pronounced in the animals that received 0.3 mg/kg of the trace element; it was 18% of the control. The number of erythrocytes and leukocytes fluctuated within physiological limits in all the groups. While it was impossible to detect any appreciable difference between the control and experimental animals with respect to the number of leukocytes, the number of erythrocytes in the experimental animals was somewhat smaller than in the control during the first 60 days. There were no changes in the color index, which is affected by blood catalase activity. The latter decreased in all the experimental animals at the end of the experiment, but the dose of nickel per se did not have any regular effect. It decreased appreciably at both the highest and the lowest doses tested.

Heart, lung, liver, spleen, and kidney sections from the experimental animals were stained with hematoxylin-eosin for microscopic examination. In contrast with the findings of Ya. M. Grushko et al., we failed to detect any gross or microscopic changes in the rat organs. Nevertheless, the experiments suggest that protracted peroral administration of nickel may not be innocuous for animals.

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GIG. SANIT. 31(8): 109 (1966)

УДК 615.777.952-032.611.341-099

О ТОКСИЧЕСКОМ ДЕЙСТВИИ НИКЕЛЯ ПРИ ПЕРОРАЛЬНОМ ВВЕДЕНИИ

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Никель как микроэлемент является составной частью организма (А. И. Войнар). В определенных малых количествах он необходим организму как катализатор ряда окислительных процессов, а при высоком содержании может оказывать токсическое действие. Известно, например, что растения, произрастающие на почве районов обогащенных никелем, приобретают уродливые формы, а у животных этих районов никель накапливается в шерсти, коже и рогах, причем у овец и коров, особенно молодняка, часто встречаются заболевания глаз в виде кератитов и кератоконъюнктивитов (С. Н. Черкинский; А. И. Войнар). Несмотря на то что имеются указания на возможную токсичность никеля при разных путях поступления в организм, вопрос о вредном действии вещества при пероральном введении остается до сих пор нерешенным. Более того, некоторые сведения как бы свидетельствуют о полной безвредности никеля при этом пути введения.

Проблема токсичности никеля при энтеральном пути введения стала особенно острой после того, как получила широкое распространение никелевая и никелированная посуда, из которой при варке может переходить в пищу значительное количество никеля (А. В. Рейслер). Но большинство авторов отмечает относительную безвредность приема этого элемента per os, ибо, пользуясь никелевой посудой, человек может получать ежедневно не более 2 мг никеля на 1 кг веса, тогда как в эксперименте никель в дозе 100 мкг/кг якобы не имеет заметного токсического эффекта. Такое мнение со всей категоричностью было выражено Леманом.

Противоречивость изложенных данных побудила нас провести длительное изучение токсических свойств никеля при пероральном пути введения в эксперименте.

С этой целью мы вводили раз в сутки с помощью зонда раствор хлористого никеля в дозах 0,06, 0,12 и 0,3 мг/кг (в пересчете на металлический никель) белым крысам в течение 13 недель. В каждую из 3 опытных групп было взято по 10 животных. Контрольная группа также состояла из 10 крыс; им вводили дистиллированную воду.

В ходе исследования контролировали общее состояние и поведение животных; их систематически взвешивали. До опыта, через 30 и 60 суток от начала его и в конце опыта определяли количество эритроцитов и лейкоцитов в периферической крови. Цветной показатель и каталазную активность крови изучали до опыта и в конце его.

В течение всего опыта животные выглядели совершенно здоровыми, активными и съедали положенный корм. Однако у крыс, получавших никель в дозах 0,12 и 0,3 мг/кг, прирост веса к концу опыта, выраженный в процентах к исходному для каждой группы, был меньше. Отставание в весе было наиболее выражено у животных, которым вводили микроэлемент в дозе 0,3 мг/кг; оно составляло 18% по сравнению с контролем. Количество эритроцитов и лейкоцитов колебалось во всех группах животных в физиологических пределах. Если при этом не удалось установить заметной разницы в количестве лейкоцитов между контрольными и подопытными животными, то количество эритроцитов у подопытных животных в течение первых 60 суток было несколько снижено по сравнению с контролем. Изменения цветного показателя не отмечалось, что касается каталазной активности крови, то она снизилась у всех подопытных животных в конце исследования, но закономерного влияния дозы никеля не обнаружено — заметное снижение каталазной активности крови оказалось как при наибольшей, так и при наименьшей из испытанных доз.

Для микроскопического исследования срезы сердца, легких, печени, селезенки и почек животных окрашивали гематоксилин-эозином. В отличие от данных, приводимых Я. М. Грушко и соавторами, мы не обнаружили макро- или микроскопических изменений органов крыс. Тем не менее опыты позволяют заключить, что длительное введение никеля per os может быть небезразличным для организма животных.

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Поступила 3/VI 1965 г.

Acta Physiol. Pharmacol. Neerl. **14** (1967) 443–447; © *North-Holland Publishing Co.*

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STIMULATION OF ENZYME ACTIVITY IN THE UTERUS OF THE GUINEA PIG BY NICKEL IONS

BY

R. H. CORMANE[1], D. SPRUIT AND J. P. KUIPER

1. INTRODUCTION

The observation that nickel ions cause a contraction of the uterus of guinea pigs (SPRUIT and KUIPER, 1967) led to an investigation into the influence of these ions on histochemically demonstrable enzymes in that organ.

Nickel usually inhibits the activity of enzymes, if any effect can be observed (VALLEE, 1961). We found, however, a characteristic stimulation of the activity of alkaline phosphatase in some circumstances. In the human uterus this enzyme has been observed to occur with variable activity and to be especially localized in the muscle fibres (OBER, 1950). It has been suggested, that the enzyme plays a role in glycogenesis and in the transport of organic substances across cell membranes (HOYNCK VAN PAPENDRECHT, 1963; HERTIG *et al.*, 1958).

2. EXPERIMENTAL

The guinea pig uteri were suspended in the apparatus according to SCHULTZ and DALE, as described before (SPRUIT and KUIPER, 1967). Some contractions were produced by the addition of histamine to the surrounding Tyrode solution. The histamine was rinsed from the uterus with Tyrode solution. A calculated amount of nickel or cobalt chloride was then added to the Tyrode solution to obtain the final concentrations shown in Table 1. When the uterus had contracted, it was allowed to relax before it was

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transferred to a tube and immersed in liquid nitrogen for quick freezing. Because the lowest nickel concentrations and all cobalt concentrations did not produce a contraction of the uterus (Table 1, *d* and *e*), a period of 5 min was allowed to pass before these uteri were taken from the Tyrode solution to be frozen. During this 5 minute period the metal ion will have been adsorbed to the uterus.

In Table 1 uteri *f* were not treated in the same way as uteri *b*

TABLE 1

Contraction and alkaline phosphatase activity of the uterus of the guinea pig with some metal ion concentrations.

	uterus stimulated by		contraction	alkaline phosphatase activity of the myometrium
	Ni^{++}	Co^{++}		
a	—	—	—	normal
b	3 mmol/l	—	+	much increased
c	0.3 mmol/l	—	+	normal
d	0.01 mmol/l	—	—	normal
e	—	3 mmol/l	—	somewhat increased
f	3 mmol/l and rinsed by Tyrode solution	—	+	much increased

and the other uteri, as they were very well rinsed with Tyrode solution to remove all nickel from the uteri. In view of the results of previous experiments (SPRUIT and KUIPER, 1967), this was achieved by washing three times immediately after the contraction, with an interval of 2 min between washes; this procedure was repeated for half an hour.

The uteri were investigated histochemically for some hydrolyzing enzymes, alkaline and acid phosphatases, AMP-ase, ADP-ase, ATP-ase and polyphosphatase, as in earlier investigations of human skin by CORMANE and KALSBEEK (1963). Only the alkaline phosphatase activity of some uteri appeared to be different from the activity of the normal histamine-stimulated uteri. In this study the method of GOMORI (1952) was applied. The results of the estimations are recorded in Table 1.

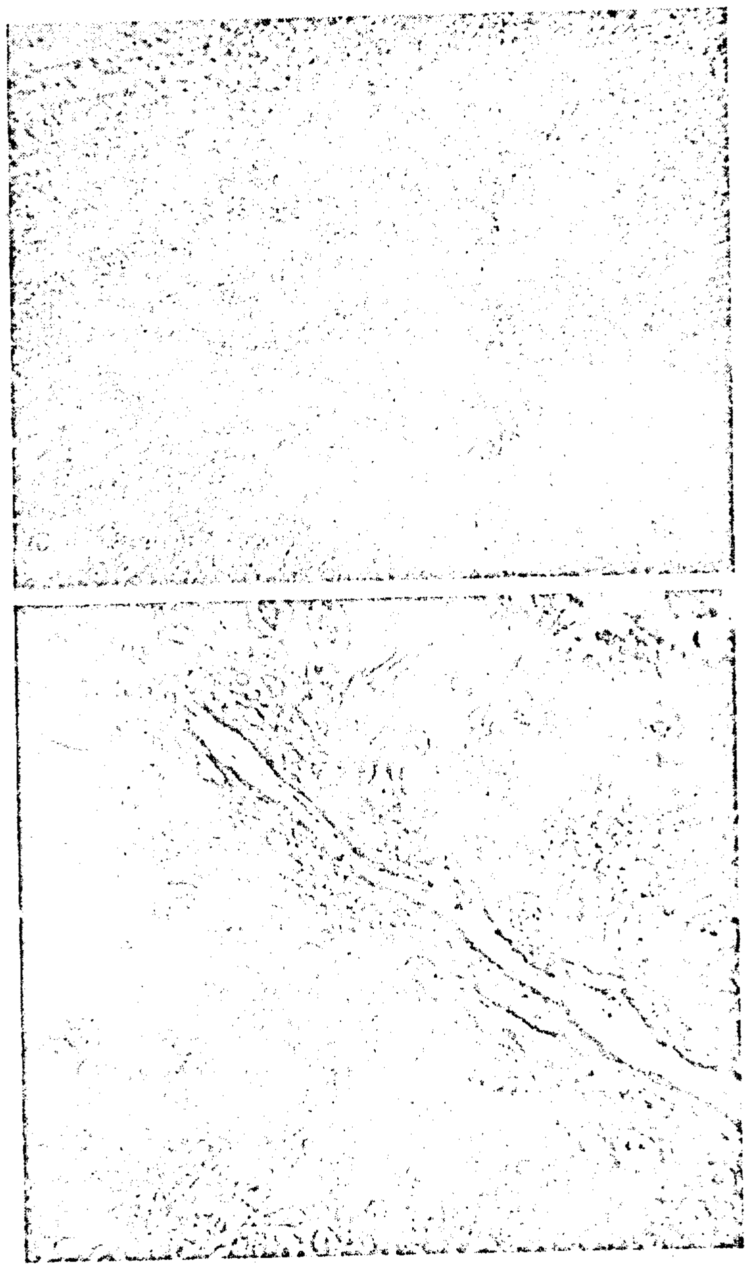
Not only was the activity of the alkaline phosphatase of the myometrium of uteri *b* and *f* different from normal but the activity and localization in the endometrium had also changed. In normal sections alkaline phosphatase activity was observed in the interstitium of the uteri only, and no activity was observed inside cells of the endometrium or at cell membranes (Fig. 1, *a*). Even the uteri, treated with 3 mmol Co/l, showed no appreciable alkaline phosphatase activity in the myometrium and the endometrium, nor was any activity of the enzyme observed at cell membranes. At the cell membranes of the glandular epithelium of uteri *b* and *f*, however, intense activity of alkaline phosphatase was seen (Fig. 1, *f*). The interior of the cells again showed no activity.

3. DISCUSSION

Because the activity of alkaline phosphatase was determined by the method of GOMORI (1952), and since during this treatment cobalt sulphide is being precipitated, the presence of nickel or cobalt originating from the experiments might possibly give rise to blackening, not originating from alkaline phosphatase activity. However, the concentrations of nickel and cobalt used in these experiments were low. Thus, a concentration of 0.3 mmol Ni/l did not blacken the sections. A concentration of 3 mmol Co/l may have blackened the sections to such an extent that the activity observed was "somewhat increased" (Table 1). Therefore, the nickel originating from the 3 mmol Ni/l solution cannot have blackened the sections appreciably. Besides, the rinsed uteri *f* were blackened as much as uteri *b*. Consequently, the really "much increased" activity of the alkaline phosphatase observed in *b* and *f* must have originated from the enzyme itself.

If the concentration of nickel ions was in excess of that required for maximum contraction and maximum adsorption, the activity of the alkaline phosphatase in the interstitium of myo- and endometrium was very much increased. It is striking, moreover, that a very strong enzyme activity was found at the cell membranes in the endometrium, where it was not present before. The presence of nickel changed the alkaline phosphatase activity very much, but only when it was present in excess.

Incubation with fresh Tyrode solution for half an hour



(experiments *f*) did not restore the low intensity of the alkaline phosphatase activity nor remove it from the cell membranes. As a consequence, the change in alkaline phosphatase activity by the transient presence of nickel is not a temporary one. This lasting enzyme activity correlates with the non-restorable contractility of the uterus after treatment with such nickel concentrations and might perhaps be correlated with variations in membrane permeability (see MEVES, 1963; HERTIG *et al.*, 1958).

SUMMARY

The alkaline phosphatase activity of the interstitium of myo- and endometrium is much increased when the uterus of the guinea pig has been exposed for a few minutes to a nickel concentration exceeding the concentration required for maximum contraction and maximum adsorption ($>$ 1 mmol Ni^{++}/l). In addition enzyme activity is found at the cell membranes of the glandular epithelium in the endometrium where it was not found before.

The alkaline phosphatase activity of uteri, exposed to Co^{++}, does not deviate from normal.

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Fig. 1. Sections of the uterus of the guinea pig. Cells coloured by hematoxylin-eosin; alkaline phosphatase activity traced by CoS (GOMORI-method).

a) uterus stimulated by histamine only; no nickel present. No alkaline phosphatase activity can be observed at cell membranes of the glandular epithelium in the endometrium (arrow).

f) uterus stimulated by 3 mmol Ni/l; immediately after the contraction the Ni was rinsed completely for ½ h. Phosphatase activity is now observed at cell membranes. For explanation see text.

Arch. Environ. Health
6(3) 381-5 (1963)

A Study of Trace Metals in Myocardial Infarction

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The possibility that trace metals may play an important part in the etiology of coronary heart disease is attracting increasing attention. Trace metals are known to be essential to certain enzyme systems, some of which are involved in the metabolism of blood lipids. Vitale and his co-workers[1] were able to reduce the deposition of lipids in the aorta and heart valves of animals fed a diet high in magnesium. Vanadium has been found to inhibit both cholesterol and phospholipid biosynthesis in animals.[2] Lewis[3] reported that the mean serum cholesterol concentration of 24 workmen exposed to vanadium was significantly lower than that of controls. Perry and Camel[4] produced a marked reduction in the serum cholesterol of 5 hypercholesteremic patients by the administration of $CaNa_2$ edetate.

The idea that trace metals may be involved in atherogenesis has also been supported by some epidemiologic evidence. Schroeder[5] reported a significant negative correlation between water hardness and mortality from cardiovascular diseases in the United States. In a further examination of various water constituents; he found magnesium and calcium to be the cations most closely related to death rates from coronary heart disease. A similar study of cardiovascular mortality in England and Wales by Morris and his co-workers[6] confirmed Schroeder's findings, except for a low correlation between coronary heart disease and magnesium. Sauer,[7] in analyzing the variation in cardiovascular mortality in metropolitan and nonmetropolitan areas of the United States, noted that, in 9 of the 10 highest rate areas, there is practically no magnesium in the drinking water, but some magnesium has been found in the drinking water of a majority of cities in the 10 lowest rate areas. Strain[2] has drawn attention to the presence of vanadium in the hard water and its absence in the soft water of certain areas of the United States. Whether the statistical associations between trace elements in drinking water and cardiovascular disease mortality are the results of cause and effect relationships or merely spurious associations has yet to be determined.

Methods and Materials

The purpose of the study reported here is to determine whether certain trace metals present in the serum are unusually elevated or abnormally absent in persons who develop a myocardial infarct by comparing the patients to a group of matched controls. Blood sera were obtained from 25 persons consecutively admitted to 2 local hospitals with a diagnosis of coronary thrombosis. Blood was drawn within 24 hours after admission. Sera were also obtained from an equal number of healthy individuals employed in the Wilmington offices of the du Pont Company. The controls were [illegible] each hospital case [illegible]. Mean [illegible] of trace

Presented at the 27th Annual Meeting of the Industrial Hygiene Foundation, Pittsburgh, Oct. 24, 1962.

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elements in the sera were made by emission spectrographic analysis, as follows:

Samples of blood serum were ashed in platinum using approximately 1 ml. of H_2SO_4. The ash equivalent to a 1 gm. sample of blood serum was placed in a graphite electrode and arced at 14 amp. d.c for 4 minutes. The spectra were recorded on Eastman I-o emulsion between 2400 and 3600A using the moving plate technique. A visual estimate of the intensities of the sample spectrogram is compared with standard spectrograms prepared from a series of "Spex" standards. These standards are supplied by Spex Industries, Inc., and contain 43 elements in a graphite base. The method yields answers good to about a factor of one-half to twice the actual values.

Since this method does not enable one to measure the actual value, the results can be presented only by specifying the range within which the actual value lies. Thus, "2-10" means that the level in the sera can be any value between 2 and 10 ppm. When the element is not detected, the initials ND are used. Since the technique is not sufficiently sensitive to detect values below certain levels, the limits of sensitivity are also specified. "ND<1", for example, means that the particular element was not detected but could have been either totally absent or have been present in some amount up to 1 ppm.

Subsequent to the admission of the patients, the hospital charts were reviewed by us for confirmation of the diagnosis. In 20 of the 25 cases, a diagnosis of myocardial infarction was definitely established by electrocardiographic and other evidence. In 2 cases, the diagnosis made subsequent to admission was coronary insufficiency; and in 3 others, the electrocardiograms indicated that myocardial infarction was possible but could not be established unequivocally. The analysis presented here is confined to the 20 patients with a definite myocardial infarction. The age and sex distribution of these patients is shown in Table 1.

TABLE 1.—*Age and Sex Distribution of Patients with Definite Myocardial Infarction*

Age	Male	Female
35-39	1	0
40-44	3	1
45-49	3	0
50-54	2	2
55-59	1	1
60-64	1	1
65-69	0	0
70-74	3	0
75-79	0	0
80-84	0	1
Total	14	6

Findings

To simplify the analysis and presentation of the findings, the data are shown in Table 2 in the form of 2×2 contingency tables; that is, tables which segregate the subjects into 4 categories. Two of the categories distinguish between the patients and the controls, and the other 2 divide the subjects into lower and higher levels of the trace elements in the sera.

Nickel, molybdenum, and boron were found to occur in significantly higher concentrations in the myocardial infarction patients than in the controls. The data also suggested an association with zinc. No significant differences between patients and controls were seen with respect to serum levels of silver, lead, manganese, iron, silicon, titanium, and aluminum. Magnesium was excluded from the analysis, because magnesium levels in all subjects were found to lie in the range, 20-100 ppm. This is a very wide range, however, so that the inability of the technique to measure the actual value may obscure an association if one exists.

The most striking difference between the infarction patients and controls was found in serum levels of nickel. In 19 of the 20 patients, the serum nickel concentration was in the range, 0.5-2 ppm or higher. Among the controls, only 4 were in this category. The difference between the two groups is highly significant ($P = 0.0000022$).* The normal range of nickel levels in human plasma is estimated to be 0.00 to 0.27 ppm.[8] Thus, all but one of the infarction patients had abnormally elevated nickel levels.

A more detailed breakdown of nickel levels is presented in Table 3. In addition to the data on the definite infarction cases, the table shows the levels exhibited by the 2 patients with coronary insufficiency and the 3 who had a possible myocardial infarction. It is noteworthy that nickel was not detectable in either case of coronary insufficiency. For one of the infarction cases, 2 samples of blood were sent to us, 1 for each of 2 admissions.

* Probability values were computed by Fisher's exact treatment of 2 x 2 contingency tables.

TABLE 2.—*Levels of Certain Trace Metals in Serum of Myocardial Infarction Patients and Matched Controls*

			Serum Levels in PPM			
		Total	ND <2	0.5-2 to 3-15	Pct. 0.5-2 to 3-15	*P* *
Ni	MI	20	1	19	95.0	
	Controls	20	16	4	20.0	0.0000022
		Total	ND <1.0	0.2-1 to 2-10	Pct. 0.2-1 to 2-10	*P* *
Mo	MI	20	8	12	60.0	
	Controls	20	18	2	10.0	0.0022
		Total †	ND <0.05 to 0.02-0.1	0.05-0.2 to 0.2-1	Pct. 0.05-0.2 to 0.2-1	*P* *
B	MI	18	8	10	55.6	
	Controls	18	15	3	16.7	0.035
		Total	ND <1 to 1-5	2-10 to 15-75	Pct. 2-10 to 15-75	*P* *
Zn	MI	20	14	6	30.0	
	Controls	20	19	1	5.0	0.091
		Total	ND <0.01 to 0.05-0.2	0.1-0.5 to 10-50	Pct. 0.1-0.5 to 10-50	*P*
Ag	MI	20	13	7	35.0	
	Controls	20	17	3	15.0	>0.10
		Total	ND <1 to 0.5-2	1-5 to 3-15	Pct. 1-5 to 3-15	*P*
Pb	MI	20	17	3	15.0	
	Controls	20	20	0	0	>0.10
		Total	ND <1 to 0.2-1	0.5-2 to 2-10	Pct. 0.5-2 to 2-10	*P*
Mn	MI	20	15	5	25.0	
	Controls	20	17	3	15.0	>0.10
		Total	2-10 to 5-25	10-50	Pct. 10-50	*P*
Fe	MI	20	18	2	10.0	
	Controls	20	19	1	5.0	>0.10
		Total	0.21- to 2-10	3-15 to 10-50	Pct. 3-15 to 10-50	*P*
Si	MI	20	16	4	20.0	
	Controls	20	17	3	15.0	>0.10
		Total	ND <0.1 to 0.2-1	0.5-2	Pct. 0.5-2	*P*
Ti	MI	20	16	4	20.0	
	Controls	20	17	3	15.0	>0.10
		Total	ND <1 to 1-5	2-10 to 5-25	Pct. 2-10 to 5-25	*P*
Al	MI	20	13	7	35.0	
	Controls	20	12	8	40.0	>0.10

*Two-tailed probability computed by Fisher's exact treatment of 2×2 contingency tables.
† The samples of sera for 2 patients were too small to permit a determination of boron levels.

TABLE 3.—*Nickel Levels in Serum of Heart Disease Patients and Matched Controls*

Nickel (ppm)	All Heart Disease	Controls	Coronary Insufficiency	Controls	Possible Myocardial Infarction	Controls	Definite Myocardial Infarction	Controls
ND <1	2	20	2	2	0	2	0	16
ND <2	2	0	0	0	1	0	1	0
0.5-2	2	1	0	0	0	0	2	1
1-5	8	2	0	0	0	1	8	1
2-10	11	1	0	0	2	0	9	1
3-15	0	1	0	0	0	0	0	1
Total	25	25	2	2	3	3	20	20

On the first admission, the electrocardiogram was equivocal; but on the second admission, one month later, a diagnosis of myocardial infarction was definitely established. It is interesting that no nickel was detected in the blood drawn on the first admission, but a level of 2-10 ppm was obtained from the blood drawn on his second admission when there was no doubt that an infarction had developed. The data we have on patients with coronary heart disease without infarction, although fragmentary, do suggest that high nickel levels are associated with infarction but not with other manifestations of coronary heart disease.

Less striking but significant differences between patients and controls were seen in serum levels of molybdenum and boron. Detectable amounts of molybdenum were found in 12 of the 20 infarction cases and in only 2 of the 20 controls ($P=0.0022$). In 2 infarction cases, the blood samples were too small to permit a determination of the boron levels. Of the 18 infarction samples analyzed, 10 showed high levels, whereas of the 18 controls high levels were found in only 3 ($P=0.035$).

Comment

Although we observed only a small sample of infarction patients and controls, the differences in serum nickel levels between the 2 groups is so large and the probability of the difference occurring by chance alone so small that further investigation of the role that nickel might play in myocardial infarction is easily justified.

A fundamental question that is raised by our observations is whether the high levels of serum nickel are a consequence of infarction or whether they are involved in the processes that lead to infarction. If the former is true, it is possible that an enzyme containing nickel is discharged into the serum following an attack of myocardial infarction. That high levels of nickel are normally present in the cardiac muscle [8] suggest that large amounts of nickel may enter the blood stream as a result of myocardial damage. Under these circumstances, serum levels of nickel may serve as a new diagnostic indicator of acute myocardial infarction.

If nickel is involved in the etiology of infarction, its role may be that of precipitating a reaction on an atherosclerotic plaque. There is also the possibility that nickel may be involved in the clotting mechanism. It has been shown that the chelating agent edetate prolongs the prothrombin time.[9] This effect is not due to the removal of calcium and magnesium, even though both of these ions are known to be involved in the clotting process. Nickel, however, is the metal which stabilizes the labile factor; and the effect of edetate on the prothrombin time arises from its chelation of nickel. Theoretically, if a chelating agent can be used to "chelate" excess nickel, it may, thereby, have a place as a preventive or therapeutic device.

Ordinarily, serum levels of trace metals in myocardial infarction are obtained after the attack, but if these metals are involved in the etiology of the disease it is essential that we know what these levels were prior to the attack. To determine whether certain metals are a "risk factor" in myocardial infarction, it is necessary to conduct a prospective type study. In this kind of investigation, a cohort consisting of a large number of healthy individuals, free of known coronary heart disease, would be chosen for observation. Trace metal concentrations in the serum would be measured on each subject. Then, the cohort would be observed for a specified period of time to measure the incidence of myocardial infarction according to various levels of the trace metals. Studies of this type have been conducted to determine the relation of coronary heart disease to blood pressure, serum cholesterol, body weight, smoking habits, alcohol consumption, and physical activity. The addition of trace metals to the current list of risk factors is worth consideration.

Summary

Sera drawn from 20 hospital patients within 24 hours after admission for myocardial infarction and sera from 20 controls matched to the patients by age and sex were subjected to spectrographic analysis to measure serum levels of the following trace metals: nickel, molybdenum, boron, zinc, silver, lead, manganese, iron, magnesium, silicon, titanium, and aluminum.

Nineteen of the 20 infarction patients, but only 4 of the 20 controls, exhibited abnormally high levels of serum nickel. The difference is highly significant. The infarction patients also showed significantly higher serum levels of molybdenum and boron. There was, in addition, a suggestion of higher levels of serum zinc in the patients. Differences between the 2 groups in serum levels of the other trace metals are well within the limits of sampling error.

Whether high levels of serum nickel are a consequence of myocardial infarction or involved in its etiology is not known. Evidence has been cited to support both hypotheses.

We wish to express our appreciation to Sr. M. John Therese and Dr. J. W. Abbiss, of the St. Francis and Memorial Hospitals, respectively, Wilmington, Del., for supplying the blood serum for the myocardial infarction cases, and to Mr. W. Koniecki, B.S., M.S., of our Medical Division Laboratory, who loaned assistance in this study.

The trace metal analyses were done at the du Pont Experimental Station by Samuel F. Ford, B.S., under the direction of Drs. J. R. Peterson and E. A. Abrahamson.

Dr. A. J. Fleming, Medical Director, du Pont Company, gave valuable guidance in this study, and we wish to both acknowledge and extend appreciation for the cooperation extended.

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J. Occupational Med
5, 71-9 (1963)

Journal of Occupational Medicine

The Role and Potential Role of Trace Metals in Disease

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OUR INTEREST in the study of trace metals in disease, especially in heart disease, was precipitated by a series of reports, including the works of Dr. Henry Schroeder of Vermont, who had noted a Japanese study, and of Dr. J. N. Morris of England, all of whom related water hardness to heart disease;[1-4] the studies of Dr. W. Strain of Rochester, N. Y., who observed large geographic variations in death rates from cardiovascular disease, possibly associated with minor element deficiencies, especially vanadium and zinc;[5] and the reports of Dr. C. Lewis, also of Rochester, N. Y., which indicated that vanadium workers had significantly lower cholesterol levels than those of controls.[6,7] Finally, the importance of trace elements in maintaining health in plants and animals is well known and still under intensive investigation.[8]

Figure 1, "Metals in Health and Disease," depicts a summarized role of some of the various metals. It is becoming increasingly obvious that metals are assuming a greater importance in the understanding of bodily functions, both in health and disease.

The variety of actions, understood and theorized, is brought forth in this brief summary. Many investigators believe that metals may hold the key to metabolic processes not now understood or understood only incompletely.

Many enzymes, which are modified proteins, require for their activity the presence of small molecules which are heat stable.[9] These are called co-enzymes. Some of these are relatively simple substances which have now been identified as metal ions, particularly magnesium, iron, zinc, and copper. Others are thought to be much more complex organic molecules derived primarily from vitamins, examples being pyridine nucleotides which are formed from thiamine, riboflavin, or nicotinamide. Heavy metals may act adversely by combining with free —SH groups, thus denaturing proteins and inactivating enzymes. Some of these are highly selective and seem to have a particular affinity for certain tissues or proteins. One metal can displace another in a biologic system and inactivate it.

Approximately 700 enzymes have been identified in animals, plants, and microbes, but only about 20 have been extensively studied in human sera.[10] With this in mind, it is reasonable to believe that additional study and investigation of these and other enzymes, particularly the metalloenzymes, might shed valuable information about various disease processes which are now

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Submitted for publication Oct. 12, 1962.

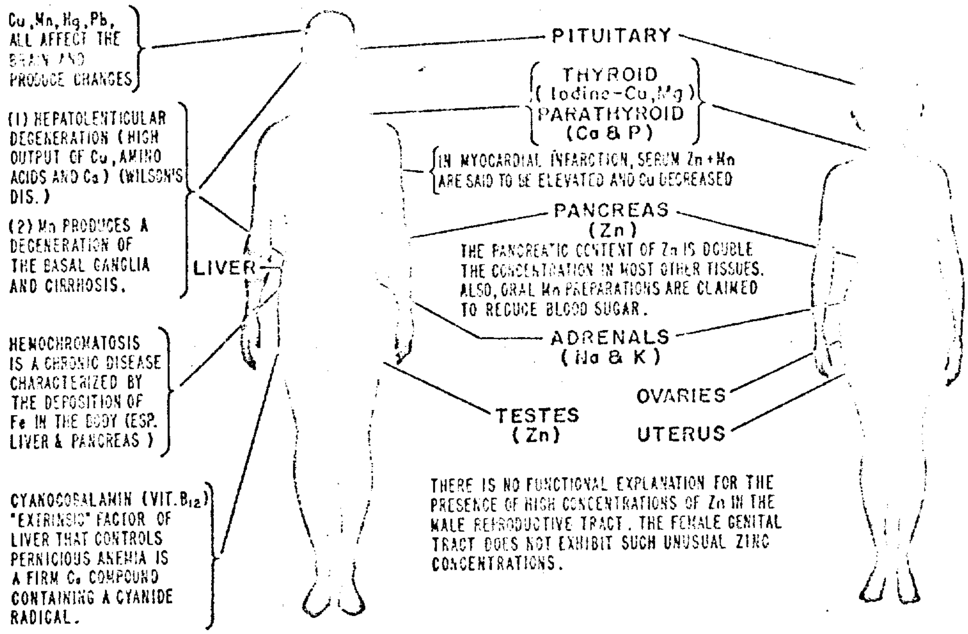

Fig. 1. Metals in health and disease.

either poorly understood, or not understood at all.

Of the 60 elements found in living things, not all are essential. Notable metals among this nonessential group are gold, aluminum, fluorine, and silicon. Although the inclusion of fluorine in this group may lead to some question because of its established effect in the prevention of dental caries, it would seem, however, that its inclusion is justified because the body can subsist in an otherwise normal state without fluorine. Actually, we do not have accurate knowledge as to which elements are and are not essential, due to the inadequacies of testing for trace metals. In planning diets for these studies, a diet distortion occurs and other foodstuffs are concomitantly omitted.

The physiological need of sodium and potassium in bodily functions is so well known as to need no review. Perhaps less well known and appreciated, however, is the place of magnesium in health and disease, about 20 gm. of which is the average amount found in the human body, and the progressive depletion of which leads to well-described signs and symptoms. See Table 1.

It has been said that magnesium and calcium are antagonistic,[11] and it has even been proposed that magnesium for this reason should be added to table salt[12] on the supposition that magnesium, which some believe to be often deficient in older people, may displace the calcium deposited in atherosclerotic areas. Magnesium deficiency is thought to be common in chronic alcoholism and may be a factor in the causation of delirium tremens.[13, 14]

Previous studies of the biological function of minerals have established well-known roles for such minerals as iron, iodine, copper, cobalt, zinc, molybdenum, and manganese. More recently attention has been directed to the possible role of rarer or less understood trace metals such as nickel, vanadium, selenium, aluminum, rubidium, cadmium, boron, cerium, and cesium.

Table 2 summarizes briefly the possible functions of trace metals in the human body.

Table 1. Well-Studied Metallic Ions

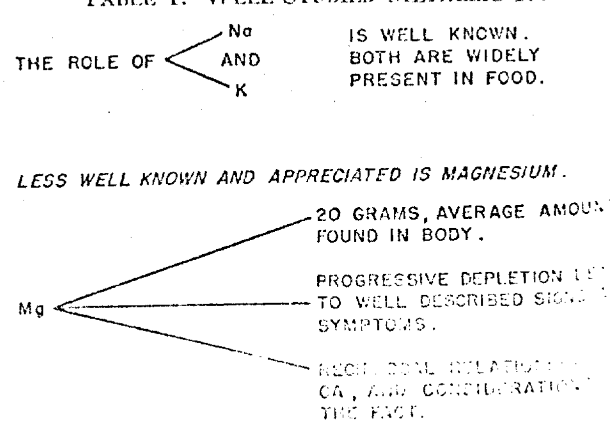

TABLE 2. TRACE METAL FUNCTIONS

- PART OF PROTEIN COMPLEX OF HORMONES AND VITAMINS:
  - EXAMPLES:
    - I AND EFFECT OF Cu, Mg ON THYROID HORMONE
    - COBALT - VIT. B_{12}
- ACTIVATION OR INACTIVATION OF ENZYMES (METALLO-ENZYMES)
- FACILITATION AND EXCHANGE OF O_2 AND CO_2
- UNKNOWN ROLES

IRON AND MAGNESIUM

PLAY A SIMILAR ROLE IN HEMOGLOBIN (left) AND CHLOROPHYLL, PARTIAL STRUCTURES OF WHICH ARE SHOWN IN HIGHLY SCHEMATIC FORM.

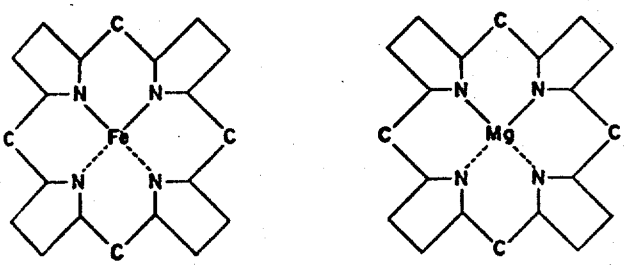

Fig. 2. (From McElvoy, W. D., and Swanson, C. P.[8])

Figure 2 depicts in highly schematic form the structural relationship of hemoglobin and chlorophyll. The similarity in schematic structure is quite obvious, with the fundamental and very slight difference being that hemoglobin contains within the formula the element iron, whereas in the chlorophyll structure, magnesium replaces iron.[8] It is further revealing and important to note that another very vital material, namely vitamin B_{12}, also has similarity to a lesser extent in structure, and again one of the prime differences being that cobalt assumes the spot held in the other two structures by iron or magnesium. The importance of this relationship is appreciated by a consideration of the fact that here we have three metals, namely iron, magnesium, and cobalt, all concerned with the role of blood in health and disease. Chlorophyll is essential in the transport of oxygen to the blood. One naturally wonders what other trace metals, the functions of which are not understood, might likewise play an essential role in cell metabolism.

The additional importance of iron in bodily health is appreciated by another study done by us which compares the frequency of elevated blood pressure by hemoglobin concentration among 1584 men, 40–64 years of age, as shown in Fig. 3. There seems to be a strong and significant relationship with the level of blood pressure and the level of hemoglobin concentration.

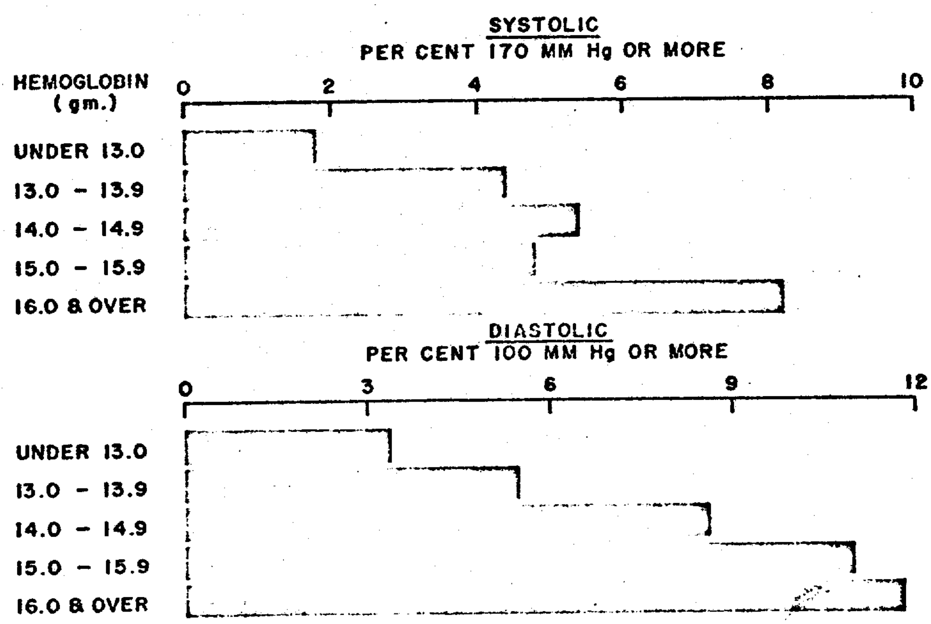

Fig. 3. Frequency of elevated blood pressure by hemoglobin concentration among 1584 men 40–64 years of age.

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TABLE 3. MEAN SERUM LEVELS OF SODIUM, POTASSIUM, MAGNESIUM, AND CALCIUM AMONG MEN WITH CERTAIN CHRONIC DISEASES

	NO. CASES	MEAN (mg. %)			
		SODIUM	POTASSIUM	MAGNESIUM	CALCIUM
ALL PERSONS	1,573	321.9	18.5	2.02	10.08
MYOCARDIAL INFARCTION	33	322.0	18.2	2.00	10.10
HYPERTENSION	224	323.0	18.4	2.03	10.16
PEPTIC ULCER	20	317.0	18.5	2.05	9.93
GALL BLADDER DISEASE	41	323.0	19.0	2.08	10.33*
DIABETES	9	321.7	18.7	2.03	9.97
ARTHRITIS	75	323.1	18.8	2.02	10.11
CATARACTS	18	324.4	19.3	2.12	10.42

*DIFFERENCE FROM MEAN FOR ALL PERSONS SIGNIFICANT AT .05 LEVEL.

TABLE 4. HISTORY OF GALLBLADDER DISEASE—CALCIUM LEVELS

UNDER 9.0	1
9.0 – 9.2	1
9.3 – 9.5	5
9.6 – 9.8	6
9.9 – 10.1	6
10.2 – 10.4	9
10.5 – 10.7	0
10.8 – 11.0	3
11.1 – 11.3	2
11.4 – 11.6	6
11.7 +	2
	41

NORMAL RANGE 8.7 – 11.5

TABLE 5. HUMAN URINE OF HYPERTENSIVES*

- INCREASED AMOUNTS OF:
  Zn – V – Ni
  Mn – Pb – Sn
- A FORTYFOLD INCREASE IN:
  – Cd –
- NORMAL AMOUNTS OF:
  Mo – Cr
  Ti – Ag

*(AFTER SCHROEDER, H.A.; POSSIBLE RELATIONSHIPS BETWEEN TRACE METALS AND CHRONIC DISEASE; METAL-BINDING IN MEDICINE; SEVEN, M.J. AND JOHNSON, L.A., J.P. LIPPINCOTT CO., PHILA., PA., p. 65)

On the other hand, the mean serum levels of sodium, potassium, magnesium, and calcium among men with certain diseases, as shown in Table 3, indicates a lesser significance between these metals and the diseases studied.

Perhaps when one has the opportunity to study these same diseases and the relationships thereto with some of the rarer metals not previously investigated, a more definite association may be found.

The frequency distribution of the calcium levels among 41 persons with a history of gallbladder disease (Table 4) shows a relatively large number of persons at the upper end of the distribution. Although the numbers are small, they do suggest some relationship between calcium and gallbladder disease.

In a previously reported study, Schroeder[15] has indicated the variation in mineral excretion in the urine of hypertensive individuals. These results are reproduced in summary form in Table 5.

There is no explanation now available for these findings.

In another study now nearing completion and publication, we have found a significant statistical relationship between the level of nickel, molybdenum, and zinc, in that order, in the blood serum of acute myocardial infarction cases when compared to a group of matched controls.

Figure 4 depicts in summary form some of the mineral studies which stimulated our interest in the possible role of trace metals in the production of myocardial infarction.[6, 16, 18]

We were primarily looking into zinc, due to the work of Vallee,[19] and were somewhat surprised from the results which indicated a possible connection between the serum level of nickel, molybdenum, and boron. Tables 6 and 7 depict

Fig. 4. Trace metals and heart disease. Nickel levels in our own study.

TABLE 6. LEVELS OF CERTAIN TRACE METALS IN SERUM OF MYOCARDIAL INFARCTION PATIENTS AND MATCHED CONTROLS

		Total	Serum levels in ppm ND < 2	Serum levels in ppm 0.5-2 to 3-15	Pct. 0.5-2 to 3-15	P*
	MI	20	1	19	95.0	.0000022
	Controls	20	16	4	20.0	

		Total	ND < 1.0	0.2-1 to 2-10	Pct. 0.2-1 to 2-10	P*
	MI	20	8	12	60.0	.0022
	Controls	20	18	2	10.0	

		Total**	ND < 0.05 to 0.02-0.1	0.05-0.2 to 0.2-1	Pct. 0.05-0.2 to 0.2-1	P*
	MI	18	8	10	55.6	.035
	Controls	18	15	3	16.7	

		Total	ND < 1 to 1-5	2-10 to 15-75	Pct. 2-10 to 15-75	P*
	MI	20	14	6	30.0	.091
	Controls	20	19	1	5.0	

*Two-tailed probability computed by Fisher's exact treatment of 2 x 2 contingency tables.
**The samples of sera for two patients were too small to permit a determination of boron levels.

…ese in summary form. Table 8 gives the age …d sex distribution of these cases.

The results obtained by studying the serum …kel determinations prompted us to review the …ssible functions and data on nickel to better …derstand these results.[20, 21] Table 9 gives in …mmary form some data on nickel, which bear …ossible relationship to this study.

We were then curious to see what, if any, stud… were done on nickel determinations in various …ds. Schoeder *et al.*[27] reported in 1961 an …alysis of the nickel content in various foods, which we reproduce in summary form, particularly with the foods or agents having the highest nickel content. These are to be found in Tables 10 and 11.

One naturally will wonder whether it might possibly be the nickel in hydrogenated fats, rather than the relationship of saturated to unsaturated fats, per se, in this respect, which is the etiological agent, or a precipitating factor, in the production of myocardial infarction.

Nickel is said to be one of the relatively nontoxic trace metals which are found in the tissues

TABLE 7. NICKEL LEVELS IN SERUM OF HEART DISEASE PATIENTS AND MATCHED CONTROLS

Nickel (ppm)	All Heart Disease	Controls	Coronary Insufficiency	Controls	Possible Myocardial Infarction	Controls	Definite Myocardial Infarction	Controls
ND<1	2	20	2	2	0	2	0	16
ND<2	2	0	0	0	1	0	1	0
0.5-2	2	1	0	0	0	0	2	1
1-5	8	2	0	0	0	1	8	1
2-10	11	1	0	0	2	0	9	1
3-15	0	1	0	0	0	0	0	1
Total	25	25	2	2	3	3	20	20

TABLE 8. AGE AND SEX DISTRIBUTION OF PATIENTS WITH DEFINITE MYOCARDIAL INFARCTION

AGE	MALE	FEMALE
35 - 39	1	0
40 - 44	3	1
45 - 49	3	0
50 - 54	2	2
55 - 59	1	1
60 - 64	1	1
65 - 69	0	0
70 - 74	3	0
75 - 79	0	0
80 - 84	0	1
TOTAL	14	6

of man, and in this respect ranking with the essential elements, iron, cobalt, copper, and zinc.[22] Its physiological role, if any, has not been delineated; but it has been said repeatedly that there has never really been a concerted effort made to determine same. Nickel is quite an ina[illegible] metal, but its compounds are active physio[illegible] cally. Nickel may be in many foods in a [illegible] similar reactively to the simpler inorganic [illegible] compounds. Nickel is thought to have some of reciprocal relationship with cobalt and is a metal which deserves more clinical study.

Studies indicate that the chelating agent EDTA prolongs the prothrombin time.[23] It has been shown that this effect is not due to the removal of calcium and magnesium, even though both of these ions are known to be involved in the clotting process. Nickel, however, is the metal which stabilizes the labile factor, and the effect of EDTA on the prothrombin time arises from its chelation of nickel.

The process of chelation in general is nicely diagrammed by Dr. G. H. Berryman of Abbott Laboratories and reproduced in Fig. 5.

There are at least three possible theories to explain the action of trace metals in general. These are found in Table 12.

TABLE 9. DATA ON NICKEL

1. A HIGH CONCENTRATION OF NICKEL IS FOUND IN HEART MUSCLE, LUNG, BLADDER, SMALL BOWEL.
2. NICKEL MAY BE FOUND IN PLASMA AND R.B.C.'S. THE MEAN PLASMA LEVEL OF NICKEL IS REPORTED TO BE 0.00-0.27 P.P.M. (IN ONE STUDY ONLY 11 OF 109 SAMPLES FAILED TO SHOW NICKEL.)
3. IN VITRO NICKEL ACTIVATES ARGINASE, CARBOXYLASE AND TRYPSIN. IT MAY INHIBIT ACID PHOSPHATASE.
4. NICKEL BEARS A CLOSE PHYSICAL AND CHEMICAL RELATIONSHIP TO CO.
5. AN ORDINARY DIET MAY SUPPLY 0.3-0.5 mg. OF NICKEL PER DAY.
6. AN ESSENTIAL FUNCTION OF NICKEL HAS NOT YET BEEN DEMONSTRATED, BUT NEITHER HAS A SERIOUS EFFORT BEEN MADE TO FIND ONE.

TABLE 10. AMOUNT OF NICKEL IN VARIOUS FOODS*

		MICROGRAMS PER GRAM
CONDIMENTS:	BAKING POWDER	13.40
	BLACK PEPPER	3.93
	TABLE SALT	0.35
FLUIDS:	TEA, ORANGE PEKOE	7.60
	COCOA	5.00
	CIDER	550/L
	BEER, CANNED	10/L
	MINERAL WATER, BOTTLED, ARKANSAS	12.5/L
MISCELLANEOUS:	GELATIN	4.50

* (FROM SHROEDER, BALASSA, AND TIPTON, J. CHR. DIS., VOL. 15, pp. 51-65, 1961.)

TABLE 11. AMOUNT OF NICKEL IN VARIOUS FOODS*

		MICROGRAMS PER GRAM
GRAINS:	BUCKWHEAT, SEED	6.45
	WHEATIES	3.00
	RYE, SEED	2.70
	OATS, SEED	2.60
	OATS, PRECOOKED, QUICK	2.35
	RICE, JAPANESE, UNPOLISHED	1.80
	VEGETABLE SHORTENING, HYDROGENATED	1.14
VEGETABLES:	BEANS, RED KIDNEY, DRIED	2.59
	BEET GREENS	1.94
	PEAS, SPLIT, DRIED	1.66
	BEANS, NAVY, DRIED	1.59
	LETTUCE, GARDEN, ORGANIC	1.14
	KALE, ORGANIC	1.12
SEA FOOD:	KIPPERED HERRING, CANNED	1.70
	OYSTERS, FRESH	1.50

* (FROM SHROEDER, BALASSA, AND TIPTON, J. CHR. DIS., VOL. 15, pp. 51-65, 1961.)

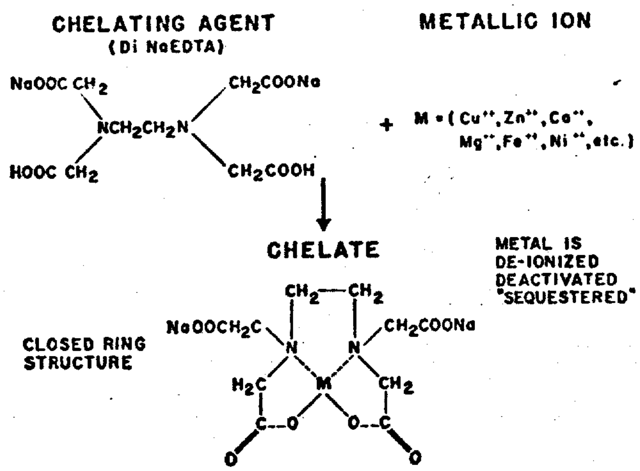

Fig. 5. (Courtesy of Dr. G. H. Berryman, Abbott Laboratories.)

TABLE 12. THREE POSSIBLE THEORIES TO EXPLAIN ACTION OF TRACE METALS

- MAY ACT AS CATALYSTS IN VARIOUS ENZYME SYSTEMS.
- AN EXCESS OR DEFICIENCY MAY ALTER NORMAL ENZYMATIC PROCESSES.
- NECESSITY OF SPECIFIC METALS IN NORMAL ENZYME SYSTEMS, AND THE EFFECT OF DISPLACEMENT BY OTHER TRACE METALS.

Table 13 contains in summary form some of the questions raised as a result of the serum nickel findings in our study.

The question might logically be asked whether or not trace metals may in some way be related to normal or abnormal cholesterol synthesis, and to the formation of atherosclerotic plaques. It is possible that these trace metals may act as catalysts in various enzyme systems, and their relative deficiency or excess or proportions may in some way be related to altering enzymatic processes. One metal may chelate another and alter enzyme or catalytic systems to the degree that abnormal physiological reactions result. It is suggested that cholesterol synthesis might be affected in some such fashion.

It may well be that metalloenzymes will play a role in medicine in the future which is comparable to that of the vitamins 25 years ago, and it is logical to consider that there may be many diseases of obscure etiology which will find their solution in the unraveling of the mysteries and the interchange of metals which are concerned with the metalloenzyme systems or co-enzyme systems within the human body.

It would be highly desirable to do nickel determinations on a large enough group of patients or individuals and study these individuals over a period of time to ascertain whether those individuals with a high, or relatively high, nickel content might be more predisposed to myocardial infarction than individuals with a negligible amount of nickel or no nickel at all in their serum. The significance of the level and duration of serum nickel deserves a detailed study. It has not yet been determined whether eating a good deal of foodstuffs with a high nickel content could conceivably precipitate an attack of myocardial infarction, or whether the change needs to occur, if at all, in the long continued ingestion of nickel-containing materials over a sufficiently long period of time to give one a high amount of nickel

TABLE 13. QUESTIONS RAISED AS A RESULT OF THE SERUM NICKEL FINDINGS

1. IS THE FINDING OF SERUM NICKEL AN ISOLATED AND INTERESTING FINDING OF NO ETIOLOGICAL SIGNIFICANCE ?
2. IS THE NICKEL DETECTED THAT NICKEL WHICH IS RELEASED INTO THE SERUM AS A RESULT OF AN ENZYME CONTAINING NICKEL BEING DISCHARGED INTO THE SERUM AFTER AN ATTACK OF MYOCARDIAL INFARCTION ?
3. IF (2) IS CORRECT, THESE FINDINGS, WITH REFINEMENTS, MAY POSSIBLY SUGGEST A NEW DIAGNOSTIC TEST FOR THE DETECTION OF AN ACUTE MYOCARDIAL INFARCTION.
4. MIGHT THE PRESENCE OF "EXCESS" NICKEL IN THE SERUM, REGARDLESS OF ITS SOURCE, PRECIPITATE A REACTION ON AN ATHEROSCLEROTIC PLAQUE TO CAUSE A MYOCARDIAL INFARCTION ?
5. IS THERE POSSIBLY ANOTHER METAL OF ETIOLOGICAL SIGNIFICANCE WHICH IS CHELATED BY A NICKEL COMPOUND, WITH THE SUBSEQUENT RELEASE OF NICKEL INTO THE SERUM AFTER CHELATION ?
6. IF THE DETECTION AND AMOUNT OF NICKEL IS OF SIGNIFICANCE, BUT IN AN UNKNOWN MANNER, MIGHT THE USE OF A CHELATING AGENT TO "CHELATE" THE EXCESS NICKEL OFFER A THERAPEUTIC OR PREVENTIVE POSSIBILITY ?

in the serum. Nor can one state with certainty whether or not the high nickel content of the serum in the individuals with coronary thrombosis was present before the attack. Studies certainly should be designed to answer these questions with certainty. The magnitude of the myocardial infarction problem, particularly as concerns industry in general, is such that these points should be adequately studied.

Summary

A review is made of background literature pertaining to the roles of metals in disease in general and in acute myocardial infarction in particular. In this study, the levels of serum nickel, molybdenum, and boron are all elevated in a group of myocardial infarction cases compared to controls. Nickel was the metal which showed the most significant elevation.

A brief discussion is given of enzymes, particularly metalloenzymes, and their potential relationship to disease.

The schematic similarity of chemical structure, save for the particular metal involved, is noted for chlorophyll (Mg), hemoglobin (Fe), and vitamin B_{12} (Co).

A relationship of blood-pressure levels to hemoglobin concentration is noted. There also seems to be a relationship between the serum calcium level and gallbladder disease.

The potential meaning of the results is discussed.

Acknowledgments

The authors wish to express their appreciation to Sr. M. John Therese and Dr. J. W. Abbiss, of the St. Francis and Memorial Hospitals, respectively, Wilmington, Del., for supplying the blood serum from the myocardial infarction cases; and to Mr. W. Koniecki, B.S., M.S., of our Medical Division laboratory, who gave valuable assistance in this study. The trace-metal analyses were done at the du Pont Experimental Station by Samuel F. Ford, B.S., under the direction of Drs. J. R. Peterson and E. A. Abrahamson. Grateful acknowledgment is made to these individuals.

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Jour. Dental Res
31(1) 129-131 (1952)

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. I

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IT IS generally believed that the production of acid by the action of microorganisms on fermentable carbohydrates in the mouth is a dominant factor in the development of dental caries. Consequently, a great deal of work has been done during the last few years in an effort to obtain a substance which would prevent or at least retard the rate of acid formation, and which could be used without danger of toxic reactions. Among the substances which have been recommended are urea,[1,2] ammonium salts,[3] vitamin K,[4] quartenary ammonium salts,[5] and penicillin.[6]

At this time we wish to report on the marked inhibitory action of several metallic salts, especially those of copper and nickel, on the production of acid by saliva containing either glucose or sucrose. Saliva was collected by paraffin stimulation and sucrose added to give a 5 per cent concentration. Aliquots were then transferred to test tubes and various amounts of a solution of the salt being studied were added to all but the control tubes. All were then made up to the same volume by the addition of the necessary amount of water. The copper salts, which are insoluble in water, were dissolved in a small amount of 5 per cent monoethanolamine and then diluted with 9 volumes of water. The maximum amount of this solution added to 9 ml. of the saliva-sucrose solution was 1 ml., thus making a final maximum monoethanolamine concentration of only 0.05 per cent. The control tubes of these experiments contained an amount of monoethanolamine equal to the maximum present in the experimental tubes. All tubes were incubated at 37° C. and their pH values were determined with a Beckman pH meter at intervals thereafter. A number of determinations were run on each compound and the results obtained were in general agreement; however, in order to conserve space, the results of only one experiment per compound are reported. Glucose was substituted for sucrose in a few experiments and comparable results were obtained. A number of other copper salts besides those reported in Table I have been been studied and all showed the same general inhibition when compared at the same copper level. These included copper butyrate, caprylate, oleate, stearate, 9-hendecynoate, 2-dodecynoate, salicylate, benzoate, mandelate, cinnamate, and ethyl acetoacetate.

It will be seen from the results that a copper concentration of 3 to 4 mg. per 100 ml. of saliva was sufficient to completely prevent acid formation, while a concentration of 0.25 mg. per 100 ml. gave fairly marked inhibition. Un-

Received for publication, June 13, 1951.

TABLE I

EFFECT OF COPPER SALTS UPON ACID PRODUCTION IN SALIVA

TIME OF INCUBATION (HRS.)	CONCENTRATION OF COPPER MG./100 ML.							SUBSTANCE USED
	4 (pH)	3 (pH)	2 (pH)	1 (pH)	0.5 (pH)	0.25 (pH)	0 (pH)	
22	7.9	7.5	7.6	6.3	5.2	4.7	4.3	Copper acetate
48	7.7	7.6	7.5	4.5	4.4	4.4	3.9	
24	–	7.6	7.3	6.3	5.7	5.0	4.3	Copper propionate
52	–	7.7	6.0	4.5	4.4	4.3	3.8	
24	–	8.4	8.2	6.9	6.2	4.9	4.5	Copper laurate*
48	–	8.4	8.1	5.3	4.9	4.6	4.0	
24	7.3	7.4	7.3	6.2	5.3	4.9	4.4	Copper salicylate
48	7.8	7.5	6.8	5.1	4.6	4.4	4.1	
22	7.9	7.7	7.6	6.3	5.2	4.8	4.3	Copper sulfate
48	7.8	7.8	5.4	4.4	4.2	4.2	3.8	
24	7.5	7.3	7.3	6.2	5.1	4.9	4.4	Copper chloride
48	7.7	7.6	7.0	4.7	4.4	4.3	4.1	
24	8.1	7.9	6.9	5.6	5.2	4.7	4.2	Copper saccharinate
48	8.1	7.9	6.1	4.5	4.5	4.3	3.8	

*This was dissolved in a weak solution of monoethanolamine.

recorded experiments with several copper salts showed that concentrations as low as 0.1 mg. of copper per 100 ml. exerted demonstrable inhibitory action, the effect being especially evident during the first 24 hours of incubation.

Nickel salts have also been studied rather extensively and found to be in general almost as active as copper salts. A few of these results along with some obtained on other metals are shown in Table II. It will be seen that silver, gold, and mercury salts are quite active, comparing favorably to copper and nickel. Magnesium, cobalt, manganese, aluminum, iron, and chromic salts were found to have little or no activity. Platinum and palladium showed moderate activity, being superior to zinc but below that of copper.

The results indicate that the inhibitory effect on acid production is primarily a function of the metal content. It is, however, possible that some metal derivative may be found in which this is not the case.

TABLE II

EFFECT OF VARIOUS METALLIC SALTS UPON ACID PRODUCTION IN SALIVA

TIME OF INCUBATION (HRS.)	CONCENTRATION OF METAL MG./100 ML.					SUBSTANCE USED
	6 (pH)	4 (pH)	2 (pH)	1 (pH)	0 (pH)	
23	7.0	6.9	6.7	6.8	4.1	Nickel propionate
51	7.1	6.7	5.5	4.9	3.7	
24	7.2	7.4	7.5	7.2	4.2	Nickel chloride
48	8.0	8.1	8.0	6.1	4.0	
24	7.3	7.4	7.4	6.7	4.4	Nickel iodide
50	7.6	7.5	7.3	5.3	4.0	
24	7.6	7.6	7.6	7.5	4.2	Silver nitrate
48	8.1	8.1	8.1	8.1	4.0	
22	7.6	7.4	7.4	6.8	4.5	Gold chloride
43	7.3	6.5	6.5	6.0	4.1	
22	7.8	7.8	7.4	7.2	4.5	Mercuric chloride
43	7.9	8.0	7.4	6.8	4.1	
24	5.2	5.0	4.5	4.2	4.2	Zinc sulfate
48	4.7	4.5	4.2	4.0	3.9	
22	4.5	4.5	4.5	4.5	4.5	Magnesium chloride
43	4.2	4.2	4.2	4.2	4.1	

SUMMARY

The effect of various metallic salts upon acid production in saliva containing sucrose has been studied. Copper, nickel, gold, silver, and mercury salts exerted the greatest inhibitory action on acid production. Magnesium, cobalt, manganese, aluminum, iron, and chromic salts showed little or no activity. Copper at a concentration of 0.25 mg. per 100 ml. of saliva containing sucrose definitely inhibited acid formation while a concentration of 3 to 4 mg. per 100 ml. of saliva caused complete inhibition of acid production.

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Jour. Dental Res.
31(1) 132-136(1952)

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. II.

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IN THE first paper of this series we have reported the marked inhibitory action of various metallic salts, including those of copper and nickel, on the formation of acid in saliva containing either glucose or sucrose.[1] The present study deals with (a) the effect of washing the mouth and brushing the teeth with an aqueous solution of these agents on the production of acid by subsequent saliva collections after addition of sucrose and incubation at 37° C., and (b) the apparent bactericidal action of copper and nickel salts on the acidogenic organisms in saliva.

EXPERIMENTAL

Effect of Mouth Washing and Teeth Brushing.—These results are divided into two sections: (a) The effect of a single brushing and washing with the inhibitor solution; (b) the effect of repeated brushings and washings. In the first case saliva was collected with paraffin stimulation in the morning after reaching the laboratory, then the mouth was washed with some brushing of the teeth for approximately 5 minutes using three separate portions of the inhibitor solution. After an additional 3 minutes, the mouth was washed with water several times so as to remove the excess inhibitor. Saliva was collected at intervals thereafter in the usual way during a 3-hour period. Sucrose was added to all the tubes so as to give approximately a 5 per cent concentration and the tubes were incubated at 37° C. for 24 hours. Several studies were run with each inhibitor and the results obtained were always in good general agreement. The averages of the various experiments in each series are shown in Figs. 1 and 2. It will be seen from Fig. 1 that a single washing and brushing with a copper solution of only 0.0035 M (22 mg. per 100 milliliters) exerted an effect for at least 3 hours. A 0.01 M (63 mg. per 100 milliliters) solution as shown in Fig. 2 is still more effective. Copper chloride was found to have a greater activity than nickel chloride. We have found this to be the general tendency among the various copper and nickel salts studied, although the differences were not always pronounced. Studies with copper concentrations of 0.0015 M (9.5 mg. per 100 milliliters) showed good inhibition of acid production but the effect did not usually last as long as 3 hours. However, the duration of effect is considerably prolonged if the first saliva collection is postponed for several hours. This may be due to the mechanical removal during the saliva collection of inhibitor which had been adsorbed or chemically combined with organic matter in the mouth.

Received for publication, June 13, 1951.

Effect of Repeated Washings and Brushings.—The purpose of this experiment was to determine if mouth washing as already described would reduce the concentration of the acidogenic organisms of the saliva over a 24 hour period, as measured by the amount of acid produced when the saliva was incubated with sucrose. Saliva collections were made at approximately 7:30 A.M., 12 noon, 6 P.M., and 11 P.M. Breakfast was around 7:45, lunch at 12:30, and dinner at 6:30. The mouth was washed shortly after each meal and at 11:10 P.M., just before retiring. During the control period at the beginning of the experiment, the teeth were brushed and mouth washed with water instead of with the inhibitor solution. The samples collected at 6 P.M., 11 P.M., and 7:30 A.M. were kept until the 12 noon collection when sucrose was added to all and the tubes incubated together for 23 hours. Tests have shown that the pH reached by untreated saliva after addition of either glucose or sucrose was not measurably affected by the saliva standing at room temperature for 24 hours prior to addition of the sugar and incubation. It will be seen from the results which are recorded in Fig. 3 that the teeth brushing and mouth washing either caused a marked reduction in the number of acidogenic organisms in the mouth or reduced in some other way their acid-producing potential.

Bactericidal Studies.—A large amount of saliva was collected in the usual manner, thoroughly mixed and 10 ml. portions were transferred to sterilized 50 ml. centrifuge tubes, care being taken not to get the saliva on the side of the tubes. Aliquots of a standard solution of the inhibitor were added to give the desired inhibitor concentration and the contents were mixed by gentle tapping. After various periods of time, 0.5 ml. of the treated saliva was transferred with sterile precautions to a culture tube. Control tubes were similarly prepared using 0.5 ml. of the same saliva without inhibitor. The tubes were then incubated at 37° C. for the specified period of time after which their pH was measured with a Beckman pH meter. The culture tubes were prepared as follows: Saliva was collected with paraffin stimulation and sucrose added to make a 5 per cent solution. Aliquots of 9.5 ml. were transferred to test tubes, plugged with cotton and sterilized at 15 pounds pressure for 15

TABLE I

BACTERICIDAL ACTION OF COPPER AND NICKEL CHLORIDE ON THE ACIDOGENIC ORGANISMS IN SALIVA

DURATION OF INCUBATION (HOURS)	CONCENTRATION OF INHIBITOR TIMES 10^{-3} M				TIME SALIVA IN CONTACT WITH INHIBITOR
	2.0 (pH)	1.5 (pH)	1.0 (pH)	0 (pH)	
48	--	6.8	4.6	4.5	$NiCl_2$ for 15 minutes
48	--	7.9	4.7	4.4	As above for 1 hour
48	--	8.0	6.5	4.3	As above for 23 hours
48	5.5	5.1	4.7	4.4	As above for 15 minutes
48	5.9	5.1	4.7	4.4	As above for 1 hour
48	8.0	7.6	5.2	4.4	As above for 24 hours
48	--	4.9	4.9	4.5	$CuCl_2$ for 15 minutes
48	--	4.8	4.9	4.4	As above for 1 hour
48	--	7.9	8.0	4.3	As above for 23 hours
48	6.9	5.1	4.9	4.4	As above for 15 minutes
48	8.0	4.9	5.0	4.4	As above for 1 hour
48	8.0	5.3	6.9	4.4	As above for 24 hours

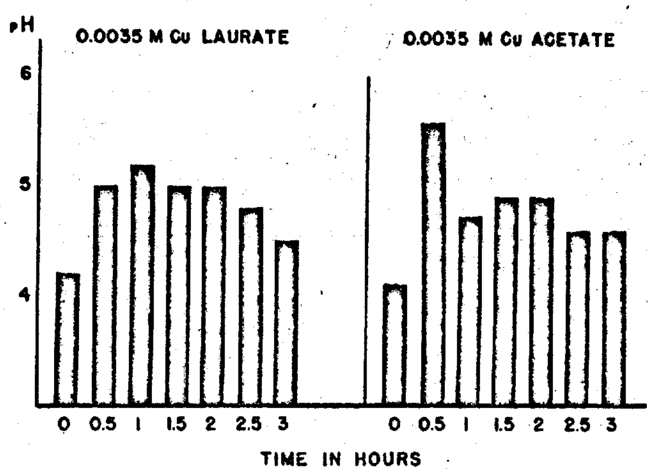
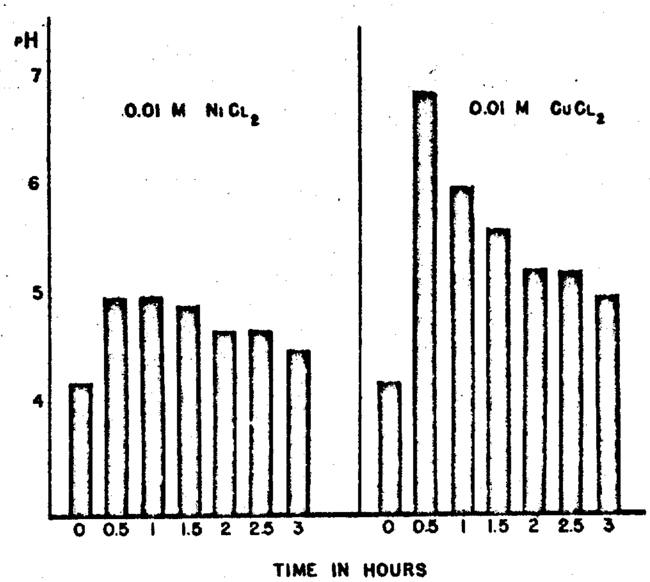

pH
0.0035 M Cu LAURATE
0.0035 M Cu ACETATE
6
5
4
0 0.5 1 1.5 2 2.5 3
0 0.5 1 1.5 2 2.5 3
TIME IN HOURS

Fig. 1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7
6
5
4
0.01 M NiCl2
0.01 M CuCl2
0 0.5 1 1.5 2 2.5 3
0 0.5 1 1.5 2 2.5 3
TIME IN HOURS

Fig. 2.

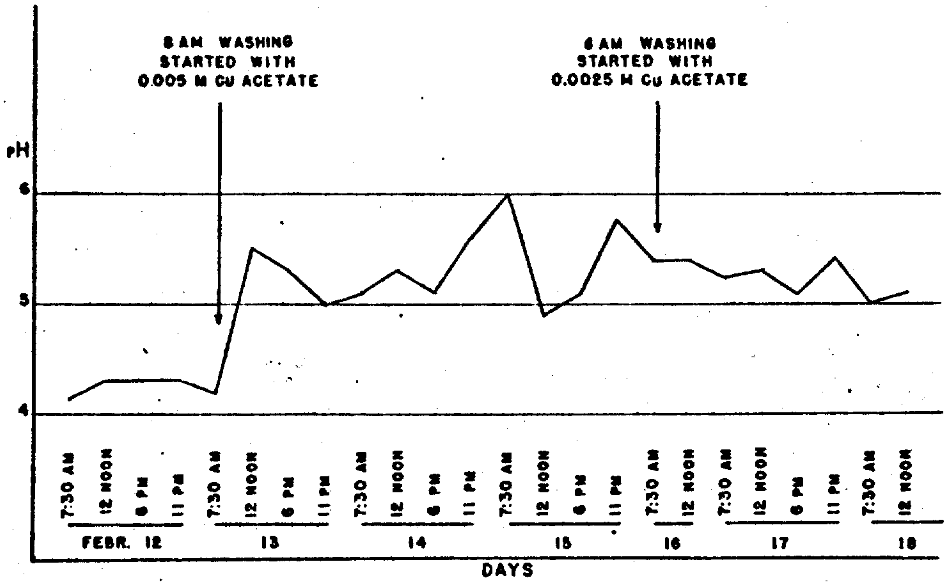

Fig. 3.

minutes. A few of the experimental results are shown in Tables I and II. It will be seen from Table I that a concentration of 0.002 M of nickel or copper chloride either destroyed the acidogenic organisms or completely inhibited in some other way their acid-producing power when exposed to it for about 24 hours. Even an exposure of 15 minutes resulted in the apparent destruction of many of the organisms. It should be noted that the maximum concentration of inhibitor in any of the culture tubes was only 0.0001 M (0.63 mg. per 100 milliliters in the case of copper). As reported in Part I of this study, this concentration although exerting a distinct inhibitory effect on acid formation is well below that necessary for complete inhibition.[1] Results obtained with copper laurate dissolved in triethanolamine are shown in Table II. The results

TABLE II

BACTERICIDAL ACTION OF COPPER LAURATE-TRIETHANOLAMINE SOLUTION OF THE ACIDOGENIC BACTERIA IN SALIVA

DURATION OF INCUBATION (HOURS)	CONCENTRATION OF INHIBITOR TIMES 10^{-3} M					TIME SALIVA IN CONTACT WITH INHIBITOR
	1.72 (pH)	.86 (pH)	.43 (pH)	.17 (pH)	0 (pH)	
47	8.0	5.6	5.3	4.6	4.1	70 minutes
48	7.8	5.9	5.0	4.9	4.3	27 hours
47	7.7	4.7	4.5	4.5	4.1	70 minutes
48	8.0	5.2	4.9	4.8	4.3	27 hours
48	7.6	5.6	4.8	4.8	4.1	1 hour
72	7.9	7.9	7.6	4.7	4.2	24 hours
96	5.4	5.0	4.5	4.0	4.0	1 hour
96	8.3	8.1	4.9	4.0	3.8	48 hours

on the whole are roughly comparable to those obtained with copper chloride. The maximum amount of triethanolamine present in the culture tubes was only 0.02 per cent, consequently neutralization of acid by this base cannot be responsible for the absence of a drop in the pH of the tubes which had been inoculated with saliva exposed to the higher concentration of inhibitor. The minimum concentration of inhibitor necessary for apparent destruction of the organisms during a 24 hour exposure varied slightly in different experiments. This difference may be due to variations in the number of organisms and the amount of proteinaceous material in the saliva.

All of the copper compounds, which have been tested, have a metallic taste and their continued use four times a day as a mouthwash in the higher concentrations cause a dark deposit along the longitudinal fissure of the tongue. This deposit, however, disappears in a few days after the mouth washing is discontinued. The saccharinate has the most pleasant taste of all the copper salts thus far tested and more work is being carried out with this preparation.

SUMMARY

Data have been presented showing that when the mouth is washed and the teeth brushed with an aqueous solution of a copper or nickel salt, there is marked reduction in the amount of acid produced in saliva samples collected over the next few hours. This effect can be maintained over a 24-hour period if the brushing and washing are repeated after each meal and again shortly before retiring. Copper or nickel concentrations of 0.002 M apparently destroy the acidogenic organisms of saliva if they are exposed to it for as long as 24 hours.

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ALLERGY TO CHROMIUM, NICKEL AND COBALT

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Patients with allergic eczematous contact dermatitis due to metals are often allergic to more than one metal. This has prompted speculation on cross sensitivity, especially between chromium, nickel and cobalt (9). Judging from recent investigations co-existing allergy to these metals is *not* due to cross sensitisation, but to the occurrence of several allergenic metals in sensitising objects (1, 9, 13, 15). For example, traces of chromium and cobalt in cement are a common cause of allergy to these metals in brick-layers (12) and cobalt in nickelplated suspenders is often responsible for nickel-cobalt allergy in female patients (2).

The intracutaneous testing method for metal allergy (3, 7) has recently been closely investigated (9, 10, 11). In intracutaneous testing, reactions can often be produced by very weak solutions. Contamination of a metal salt may therefore be an important source of error in the interpretation of the results of such tests. Marcussen found the results to a cobalt salt to be stronger in patients allergic to nickel than in those not allergic to metals (11). This could be explained by a slight contamination with nickel to which the patient reacted with an allergic reaction. In intracutaneous tests, contamination with metals from syringes and needles may also be of importance. Positive intracutaneous test reactions have been described in patients with negative epicutaneous tests to the same compound (3, 10). This may be because the intradermal test reveals weak hypersensitivity in patients in whom the amount of allergen absorbed through the epidermis is not sufficient to elicit a reaction in epicutaneous tests. Weak allergy and slight absorption of the sensitizer through the epidermis may be the explanation of single positive intracutaneous tests, not accompanied by positive epicutaneous tests (4, 9, 14). These types of reactions need not be interpreted as some special type of dermal sensitivity such as has been suggested (3).

This paper is concerned with the occurrence of isolated or combined allergy to chromium and/or nickel and/or cobalt. Most of the patients were studied with the epicutaneous testing technique. In addition, intracutaneous testing was done with highly purified nickel and cobalt in a number of patients with epicutaneous allergy to nickel and/or cobalt.

Material and Methods

Clinical material

Routine *patch tests* including nickel, cobalt and chromium were carried out on 5,416 patients (3,087 females and 2,329 males) with clinically suspected contact dermatitis between 1960 and 1964. Of these, 57 per cent were females and 43 per cent males.

Intracutaneous tests were performed with nickel and cobalt in 59 patients with positive epicutaneous tests to nickel and/or cobalt.

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Supported by grants from the Swedish Medical Research Council and from the Alfred Österlund Foundation.

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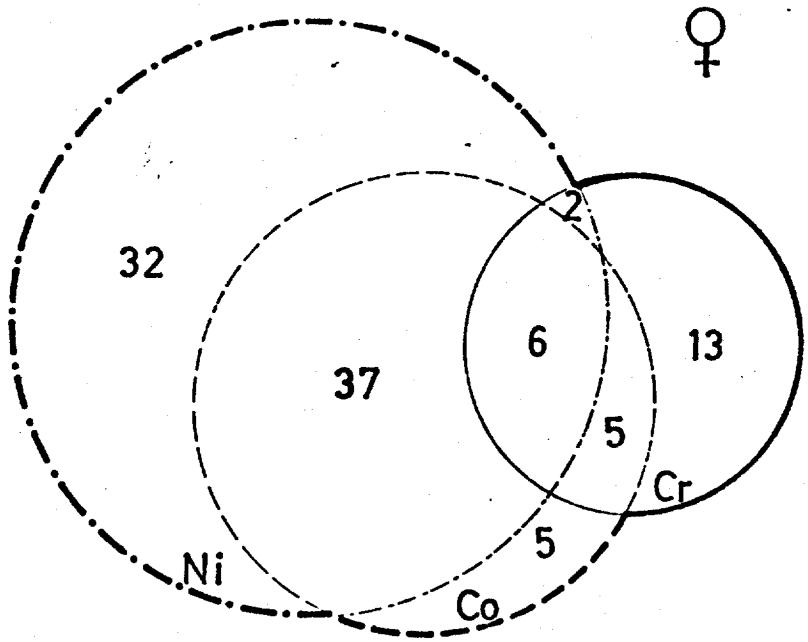

Fig. 1. Distribution of isolated and simultaneous allergies to chromium, nickel and/or cobalt in 277 females. The figures denote percentages of total number of reactions.

Test substances

Nickel

Patch test: The solution used was 2×10^{-1}M (5.25 per cent) Nickel sulfate ($NiSO_4 \cdot 6 H_2O$)[1] in distilled water.

Intracutaneous test: Nickel chloride was made from ^{58}Ni in the chemical form of NiO.[2] NiO was boiled in a reflux with concentrated hydrochloride acid in excess for three hours. After evaporation the nickel chloride was dissolved in saline to 10^{-3}M. This stock solution was then diluted to the test concentrations.

The NiO contained 0.002 per cent cobalt according to spectrochemical analysis carried out by the manufacturers. The concentrations of nickel used were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}M.

Cobalt

Patch test: The solution used was 10^{-1}M (2.38 per cent). Cobalt chloride $CoCl_2 \cdot 6 H_2O$), Analar BDH in distilled water.

Intracutaneous test: Test solutions were prepared as for nickel from cobalt metal.[3] The cobalt contained max 2 p.p.m. nickel according to spectrographic examination carried out by the manufacturers. The concentrations of cobalt used were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}M.

Chromium

Patch test: The solution used was 1.5×10^{-2}M (0.44 per cent potassiumdichromate ($K_2Cr_2O_7$)[4] in distilled water.

Testing technique

Epicutaneous test: A modification of Jadasohn-Block's technique with polythene coated almuminium patch test material (5) was used.

The volume of test solution employed was 35 microlitres/0.8 cm^2. The test substance was applied for 48 hours and the result was read after further 24 hours. A reaction with infiltration and/or vesicles or

[1] Merck, Darmstadt.
[2] Supplied by the Atomic Energy Research Establishment, Harwell, England.
[3] Supplied from Johnson, Matthey & Co., Limited, London.
[4] Hopkin & Willams.

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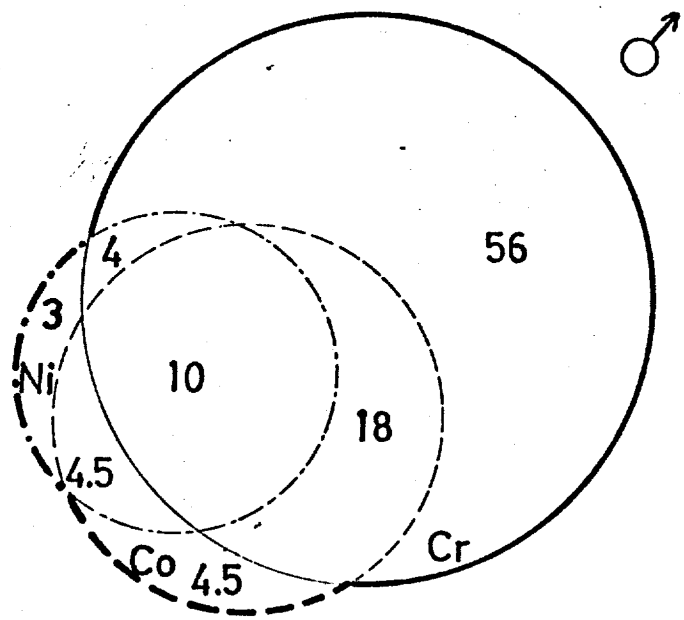

Fig. 2. Distribution of isolated and simultaneous allergies to chromium, nickel and/or cobalt in 261 males. The figures denote percentages of total number of reactions.

papules was recorded as positive, but not a pustular reaction.

Intracutaneous test. Plastic syringes and platinum needles were used. A volume of 0.1 ml of the test solution was injected superficially in the dermis of the outer side of the upper arm. The result was read after 48–72 hours. An infiltration of more than 4 mm in diameter was recorded as a positive reaction.

Table 1. *Distribution of allergic patch test reactions to chromium, nickel and cobalt*

			Number of patients		
Cr	Ni	Co	Females	Males	Females +Males
+	—	—	35	146	181
+	+	—	5	10	15
+	—	+	13	46	59
+	+	+	18	27	45
—	+	—	89	8	97
—	—	+	14	12	26
—	+	+	103	12	115
			277	261	538

Results

Epicutaneous tests

Of the 5,416 patients tested epicutaneously, 538 (277 females and 261 males) or 9.9 per cent were found to be allergic to one or more of the three metals studied. The number of positive reactions was 817. The distribution of the positive reactions to these metals in either sex is given in Table 1 and Figs. 1 and 2.

The frequency of allergy to chromium was higher among the males. Of males allergic to one or more of the metals, 88 per cent were allergic to chromium.

Allergy to nickel was more common among the females. As many as 77 per cent of the females allergic to one or more of the metals were allergic to nickel.

The relative frequency of allergy to cobalt was somewhat higher in females.

In the males allergy to chromium alone was common, but not to cobalt or nickel alone.

In the females allergy to nickel alone was common, but not to cobalt alone.

Allergy to chromium/cobalt was common in the males and to nickel/cobalt in the females, while allergy to chromium/nickel was uncommon in both sexes. The fre-

Table 2. *Intracutaneous reactions to nickel and/or cobalt in 59 patients with positive epicutaneous tests. Complete agreement between epicutaneous and intracutaneous tests*

Ni	Co	Number of patients
+	+	30
+	—	18
—	+	11

Table 3. *Reactions to intracutaneous tests with nickel and cobalt in 48 patients with positive nickel patch tests and in 41 patients with positive cobalt patch tests*

Compound	Concentration of test solution (Molarity) 10^{-6}	10^{-5}	10^{-4}	10^{-3}
Nickel	1	9	23	48
Cobalt		12	31	41

quency of allergy to chromium/cobalt/nickel was low and roughly equal in both sexes.

Intracutaneous tests

Intracutaneous testing with nickel and cobalt in 59 patients with positive epicutaneous tests to one or both of these metals gave the following results (table 2). Eighteen patients reacted positively to nickel, 11 to cobalt and 30 to both metals. When the epicutaneous test was positive, the intracutaneous test was invariably positive, too.

The strength of the allergy in the various patients is apparent from Table 3. This table shows that a 10^{-3}M solution of nickel and cobalt was sufficient to detect all cases of allergy. When a 10^{-3}M solution did not elicit a reaction, a 10^{-2}M solution never did so either. Of 19 patients who were allergic to nickel but who reacted negatively to the epicutaneous test with cobalt, none reacted positively to 10^{-2}M cobalt solution given intracutaneously, and of 11 who were allergic to cobalt but who did not react positively to nickel epicutaneously, none reacted positively to 10^{-2}M nickel solution given intracutaneously.

Discussion

The importance of allergy to metals is apparent from the fact that as many as 10 per cent of the patients tested in our series were sensitive to one or more of the three metals, chromium, nickel and cobalt.

The importance of chromium as a source of allergy among men is obvious from our series. This allergy is mainly of occupational type and could be traced to exposure of chromium among cement workers. As early as 1952 it was demonstrated that combined allergy to chromium and cobalt among cement workers was due to simultaneous contact with these metals (12). As many as 28 per cent of our allergic patients were allergic to chromium/cobalt.

Nickel allergy was more common among the women than among the men. While allergy to chromium/cobalt was more common among the men owing to occupational contact with cement, allergy to nickel/cobalt was more common among the women owing to contact mainly with metal attachments of the clothing (pins, brooches, zips, suspenders etc.). Experimental studies argue strongly against the occurrence of gross allergy between nickel and the other metals studied (15).

The low incidence of allergy to chromium/nickel in both sexes is remarkable, since stainless steel, with which both sexes come into contact, contains both chromium and nickel (usually 18 per cent Cr and 8 per cent Ni). However, chromium as a metal is not an allergen (8). Moreover, judging from clinical experience, nickel in the form it occurs in stainless steel is much less allergenic than in plated articles.

The low incidence of nickel allergy in cement workers allergic to chromium (13) can be explained by the fact that nickel occurs as a non-allergenic oxide (NiO) in cement (6). In contrast, all cobalt oxides, and therefore also those occurring in cement, are allergenic (6).

The results of the intracutaneous tests with highly purified salts in 59 patients with positive epicutaneous tests to nickel or cobalt coincided with those of the epicutaneous tests.

SUMMARY

Allergy to chromium, nickel or cobalt was demonstrated in 538 or 9.9 per cent of 5,416 patients tested epicutaneously.

Of the males allergic to metals, 87 per

cent were allergic to chromium. Of the females allergic to metals, 77 per cent were allergic to nickel. Allergy to cobalt was equally common in both sexes.

Allergy to chromium/cobalt was common in men and to nickel/cobalt in women.

Allergy to more than one of the metals studied can be explained by the co-occurrence of various metals in sensitizing products. The difference in the sex distribution of allergy to one or more of the metals can be explained by differences in exposure to sensitizing contacts.

In 59 patients allergic to nickel and/or cobalt the results of the intracutaneous testing coincided exactly with those of epicutaneous testing.

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Cancer Res. 22(2)158-162
(1962

Metal Carcinogenesis

II. A Study on the Carcinogenic Activity of Cobalt, Copper, Iron, and Nickel Compounds*

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SUMMARY

Nickel sulfide, nickel oxide, cobalt sulfide, and cobalt oxide were shown to be carcinogenic on single intramuscular injection in rats. The sulfide of each metal induced a significantly higher tumor incidence than did its oxide. Comparisons between average latent periods and between measures of tumor progression also indicated that the presence of the sulfide enhances the carcinogenic activity of these compounds. Nickel sulfate, iron and copper sulfides, and oxides failed to induce tumors in rats under these conditions.

Both C3H and Swiss mice developed tumors from single intramuscular exposure to Ni_3S_2 and to NiO; however, no tumors were induced with CoO in this species. Tumor response in mice was consistently lower than in rats, and no apparent enhancing effect of the sulfide was evidenced.

In rats, almost all tumors examined histologically were rhabdomyosarcomas, with a few fibrosarcomas, particularly among those tumors induced with NiO. Difficulty was encountered in definitely classifying the mouse tumors; whereas most appeared to be fibrosarcomas, many of these were not typical, and a few were almost certainly myomas.

It was concluded that nickel sulfide was probably the compound responsible for the carcinogenic activity of the sample of metallurgical dust originally investigated (collected from the dust flue of a nickel refinery).

The apparent specificity of nickel and cobalt compounds for striated muscle tumorigenesis is discussed.

A metallurgical powder[1] collected from the dust flue of a nickel refinery and milled to particle sizes of less than 5 μ has been shown to be a potent local carcinogen when introduced intramuscularly as a single injection in either rats or mice (3). In the same study, cobalt oxide (accounting for approximately 1 per cent of the dust) was also shown to be carcinogenic to rats but not to mice. A majority of the sarcomas examined in these initial experiments appeared to be of muscle-cell origin.

The present report deals with a determination of the components of this refinery dust responsible for its tumorigenicity, and our observations on the comparative tumorigenic activities of these and related compounds. The following six compounds, present in the original sample of refinery dust, were tested against rats: CuO, Fe_2O_3, NiO, Ni_3S_2, $NiSO_4 \cdot 6H_2O$, CoO, as were also several sulfides of copper, iron, and cobalt. Subsequently, several

* This work was supported in part by grants-in-aid of research from the National Cancer Institute of Canada.

[1] Estimated composition:

	Per cent
Cupric oxide (CuO)	3.4
Nickel sulfate ($NiSO_4 \cdot 6H_2O$)	20.0
Nickel sulfide (Ni_3S_2)	57.0
Nickel oxide (NiO)	6.3
Cobalt oxide (CoO)	1.0
Ferric oxide (Fe_2O_3)	1.8
Silicon dioxide (SiO_2)	1.2
Miscellaneous	2.0
Moisture	7.3

Received for publication July 10, 1961.

groups of mice were exposed to single intramuscular injection with those components of the refinery dust that had proved tumorigenic to the rat.

MATERIALS AND METHODS

The experimental animals used were a commercial strain of Wistar rats[2] and C3H and Swiss mice bred in our own colony. All animals were from 2 to 3 months of age when placed on experiment. Housing and care were the same as reported previously, as also was the method of preparation and administration of suspensions of the metallurgical powders (3).

As a general rule, a single 20-mg. dose of the powder under test was injected into both the left and right thigh muscles of each rat, whereas the mice received injections of 5 mg. per thigh. Exceptions to this were made for reasons of toxicity and are noted in the text where they occurred.

RESULTS

Table 1 summarizes the findings in the initial series of tests for the carcinogenicity of the compounds listed, with the rat used as the test animal.

Apparently, cupric oxide and ferric oxide, at a 20-mg. site dosage, and nickel sulfate at 5 mg/site showed no carcinogenic activity through observation periods of approximately 20 months following exposure.

Both the sulfide and the oxide of nickel induced tumors at the site of injection in a majority of the rats treated. However, marked differences in tumorigenic activity were observed between these two nickel compounds both in proportion of injection sites at which tumors developed and in average latent period. Thus, the difference between the 80 per cent tumor incidence at sites injected with Ni_3S_2 and the 41 per cent incidence with NiO is significant at the 1 per cent level (χ^2 = 16.622, df = 1, P < .01). Similarly, the 302-day latent period required on the average for NiO to induce a palpable tumor is significantly longer than the 150 days required by Ni_3S_2 ("t" = 7.855, df = 44, P < .01).

Cobalt oxide had previously been shown to be carcinogenic to the rat under these conditions of experiment (3). A further test of this compound again resulted in approximately a 50 per cent tumor response (Group 6).

The fact that injection with nickel sulfide resulted in tumors developing in 89 per cent of all the exposed animals, whereas only 66 per cent of the NiO-treated rats and 50 per cent of those

TABLE 1

LOCAL TUMOR RESPONSE OF RATS TO SINGLE INTRAMUSCULAR INJECTIONS OF METALLURGICAL COMPOUNDS

Group no. and compound	No. on exp.	Effect. no. rats*	Total inject. sites	Total tumors at site	No. tumor rats	Days to 1st tumor	Av. latent period (days)	Days on exp.	Survivors at end exp.	Miscellaneous tumors
1. CuO	32	28	56†	0				595	10	
2. FeO	32	32	64	0				601	14	
3. $NiSO_4 \cdot 6H_2O$	32	27	54‡	0				603	13	1 lymphoma 1 uterine fibroma 4 mam. fibroadenomas
4. NiO	32	32	64	26‖	21	180	302‖	595	5	1 mam. fibroadenoma 1 lymphoma
5. Ni_3S_2	32	28	45§	36‖	25	91	150‖	365	0	
6. CoO	32	24	29#	13	12	96	173	342	5	
7. CoS	30	29	58	35	28	97	196	365	1	
8. CuS	30	30	60	0				603	19	1 mam. fibroadenoma 1 reticulocytoma
9. Cu_2S	30	30	60	0				603	20	1 mam. fibroadenoma
10. FeS (47% S)	30	30	60	0**				627	21	2 mam. fibroadenomas
11. FeS(38.3% S)	30	30	60	0				627	18	2 lymphomas

* Number surviving; 90 days after treatment.
† 20 mg. right leg; 6 mg. left leg.
‡ 5-mg dose/thigh.
§ Seventeen rats treated in both thighs, eleven in one thigh only.
# Five rats treated in both thighs, nineteen in one thigh only.
‖ Differences significant at 1 per cent level.
** One rat (died 139 days) with large hematoma at injection site with indications of early sarcoma formation in thickened fibrous wall.

[2] Woodlyn Farms, Guelph, Ontario, Canada.

receiving CoO responded to the same or heavier dosages, suggested that possibly the sulfide itself might be directly concerned in this enhancement of responsiveness. To test this hypothesis, groups of 30 rats each were exposed to single intramuscular injections of the following metal sulfides:

Cuprous sulfide
Cupric sulfide
Iron sulfide (47 per cent S)
Iron sulfide (38.3 per cent S)
Cobalt sulfide

Results obtained in these tests are included in Table 1 (Groups 7–11). Copper and iron compounds again failed to induce tumors. However, the sulfide of cobalt proved markedly more active than the oxide in numbers of tumors induced, although no difference developed in average latent period.

The great majority, possible all, of tumors induced by nickel sulfide and cobalt oxide were pleomorphic, highly cellular sarcomas, obviously of striated muscle origin (Fig. 1), showing frequent metastasis to the lung and lymph nodes (Fig. 2). Although a majority of the nickel oxide-induced tumors could also be shown to be rhabdomyosarcomas (Fig. 3), about 20 per cent were classed as fibrosarcomas (Fig. 4). Several of the tumors examined from the CoS group were highly anaplastic and difficult to categorize definitely; however, here again the predominant histological type was a striated muscle tumor (Fig. 5).

Metastases were very frequent in animals of the nickel sulfide group, occurring in almost all animals autopsied (Table 2). Fifty-five per cent of the cobalt sulfide group also showed metastases, although these frequencies were progressively reduced when the primary tumor was induced by either nickel oxide or cobalt oxide.

In all cases the primary tumors, once established, tended to grow rather rapidly and often to a very large size around the site of injection. The rapidity with which the induced tumors resulted in the death of their hosts (tumor progression time) is compared in Table 3. Tumor-bearing animals whose death was attributable to causes other than tumor growth and metastasis have been omitted from these calculations. The difference of 28 days in progression time that existed between animals supporting Ni_3S_2- and NiO-induced tumors was significant ("t" = 2.219, df = 34, $P < 0.05$), as also was the difference of 29 days between average progression times of CoO and CoS-induced tumors ("t" = 3.41, df = 34, $P < 0.01$). No apparent difference existed in the rapidity with which the cobalt oxide and nickel oxide-induced tumors caused the death of their hosts.

Tumor response of mice.—Table 4 compares the tumor response of mice of two different strains to nickel sulfide and nickel oxide. The response of Swiss mice exposed to CoO at a heavier dose has been included for comparison.

TABLE 2

FREQUENCY AND DISTRIBUTION OF METASTASES IN INDUCED RAT TUMORS (MOSTLY RHABDOMYOSARCOMA)

Carcinogen	No. rats autopsied	Metastases		Distribution		
		No.	Per cent	Lung and lymph nodes	Lung only	Lymph node only
Nickel sulfide	21	20	95	14	2	4
Cobalt sulfide	29	16	55	9	1	6
Nickel oxide	20	7	35	3	2	2
Cobalt oxide	12	3	25	2	1	

TABLE 3

COMPARISON OF THE AVERAGE TIME FROM FIRST APPEARANCE OF TUMOR TILL DEATH IN RATS EXPOSED TO FOUR CARCINOGENIC METALS

Carcinogen	No. rats	Av. progression time (days)	Difference (days)
Nickel sulfide	20	55	
Nickel oxide	16	83	28*
Cobalt oxide	12	71	12
Cobalt sulfide	24	42	29†

* Significant at the 5 per cent level.
† Significant at the 1 per cent level.

The fact that both nickel compounds proved carcinogenic to mice is in sharp contrast to the inactivity of CoO, even when administered in double the dose and kept under observation for 2 years. No differences can be noted in the tumorigenic activity of the two nickel compounds in mice either in average latent period or numbers of tumor-bearing animals. However, in this species it would appear that the progression time of the NiO-induced tumors was appreciably shorter than that of Ni_3S_2 tumors (Table 4), an observation in direct contrast to the condition observed in rats (Table 3). There also appeared to be a strain difference in susceptibility to the nickel compounds, with the C3H being more refractory than Swiss on the basis of per cent tumor response. Both sexes were included in about equal numbers in the composition of these groups, with no sex differences being observed.

Exact histological classification of these mouse tumors proved to be difficult. All were sarcomas, many of which show the characteristics of a fibrosarcoma. Frequent examples, however, were encountered of both NiO- and Ni_3S_2-induced tumors with the superficial features of a fibrosarcoma (Fig. 6), which on closer examination showed a lack of collagen, a considerable cellularity, and numerous oval nuclei suggestive of a myoma (Fig. 7). A few of the mesenchymal tumors of this type also showed areas of cellular pleomorphism, with giant cells that may have been of muscle-cell origin (Fig. 8). Metastases to the lung occurred only occasionally, while the lymph nodes have not been seen to be involved in mice. This absence of dissemination through the lymphatics is in marked contrast to the situation commonly observed in the rat (Table 2).

TABLE 4

CARCINOGENICITY OF NiO, Ni_3S_2, AND CoO ON INTRAMUSCULAR INJECTION INTO C3H AND SWISS MICE

Treatment				Tumor response					
Metal	Strain and no. mice	Total sites injected	Dose/site (mg.)	No. tumor animals	Per cent affected sites	Av. latent period (days)	Av. progr'n time (days)†	Duration of exp. (days)	Survivors at end of exp.
Ni_3S_2	Swiss-45	51*	5	27	53	250	131	478	8
	C3H-18	30	5	9	33	225	139	448	2
NiO	Swiss-50	100	5	33	35	287	78	476	4
	C3H-52	104	5	23	23	256	71	476	8
CoO	Swiss-46	92	10	0				751	12

* Only a few Swiss mice were injected in both thighs with Ni_3S_2, owing to its toxicity.

† Average number days from appearance of first tumor to death.

DISCUSSION

Although the findings reported here demonstrate that several compounds of both nickel and cobalt are carcinogenic, they also indicate that in all probability nickel sulfide was the effective carcinogenic agent in the original crude refinery dust investigated. This view is supported by the rapidity with which Ni_3S_2 induced tumors in the rat, the failure of CoO to induce tumors in mice, and the fact that 57 per cent of the refinery dust was made up of the sulfide of nickel.

Needless to say, direct extrapolation between the induction of rhabdomyosarcomas in the rat and the occurrence of tumors of the sinus and lung in refinery workers is not justifiable—a fact that is underlined by our observation that parenterally administered cobalt oxide, while highly carcinogenic to the rat, is apparently inactive in mice. Nevertheless, the demonstrable tumorigenic capabilities of finely powdered compounds of nickel and cobalt in laboratory animals lends further support to the view that certain such dusts in the refinery industry may constitute an industrial cancer hazard (9, 11). Sinus and lung cancer occurring in plants where nickel is produced by the decomposition of gaseous nickel compounds has been included in the list of Proscribed Diseases in Britain for many years (4). During the last decade, however, evidence has been accumulating that this restriction of the industrial cancer hazard to the extremely finely divided nickel liberated from carbonyl, $Ni(CO)_4$, is probably not justifiable (1, 4).

The reports of Heath (7) on the carcinogenicity of metallic cobalt and particularly of the apparent specific affinity of this metal for muscle in the rat are corroborated by our own findings with both the oxide and sulfide of cobalt. Of even more interest is the fact that this peculiar selectivity for muscle has, in our experience, been shown to be particularly pronounced when the sulfides of nickel or cobalt were used as the carcinogenic agent. Hueper (10) has reported a wide variation in histogenetic types of tumors resulting from intrafemoral injection of nickel and has concluded "... that no tissue specificity exists in relation to the carcinogenic action of nickel." This is in contrast to our observations on the rat which are strongly suggestive of a definite tissue preference by this carcinogen for striated muscle. A possible reason (other than the different injection site used) for this apparent divergence may have been the high incidence of a rather wide variety of "spontaneous" tumors in Hueper's strain of rats, as indicated from his report of ten neoplasms of at least five different histogenic types in 23 control animals. In the light of both Heath's and our own observations and the relative rarity of occurrence of striated muscle tumors, Hueper's finding of several rhabdomyosarcomas developing along the site of intrafemoral injection is of considerable interest.

It is tempting to speculate what role the sulfides themselves may play in metal carcinogenesis, particularly in view of the significant enhancement to tumorigenic activity shown by sulfides of both nickel and cobalt over that of the oxides of these metals. As neither iron nor copper sulfides induced tumors, it seems safe to conclude that the sulfides per se have no carcinogenic activity. Thus, their function, if any, must be assumed to be one of true co-carcinogenesis, possibly involving alterations in the solubility and/or binding of the metal compounds. It has been suggested speculatively (5) that the introduction of excess metal into a system of delicately balanced metals and metal enzymes might readily interfere with normal function in such a way as to lead to a specific respiratory change and mutation. Excesses of either nickel or cobalt compounds might conceivably affect the same muscle enzyme system in the rat.

Heath (8) has reported that, in an experiment still in progress, cobalt has failed to induce tumor development in mice. This supports a similar observation by us, on the basis of which we had concluded that cobalt oxide could not be the effective carcinogen in the original refinery dust investigated (3). Furthermore, in mice, tumors classifiable as definitely of muscle cell origin rarely occurred in response to nickel compounds, and the fibrosarcomas that predominated appeared to be of a rather low order of malignancy, developing relatively slowly and rarely metastasizing. No marked enhancing effect of the sulfide of nickel occurred in this species. Such differences in response to the same carcinogens underscore both the desirability of utilizing more than one species in screening and the risk of error inherent in interpolation of findings from one species to another.

The consistently lower tumor response of mice of the C3H strain to nickel compounds, when compared with the Swiss mice tested, is probably a reflection of a general or systemic tumor resistance rather than of a specific refractoriness of local tissues to the carcinogenic agent used (2).

ACKNOWLEDGMENTS

The assistance rendered by Doctors J. D. Schroder and K. V. Jubb of the Pathology Division, Ontario Veterinary College, Guelph, in histopathology and in the taking of photomicrographs, respectively, is gratefully acknowledged. The help and cooperation received from Mrs. Agnes Anderson and Mrs. N. Gohlich in both the care and routine inspection of the experimental animals are gratefully acknowledged.

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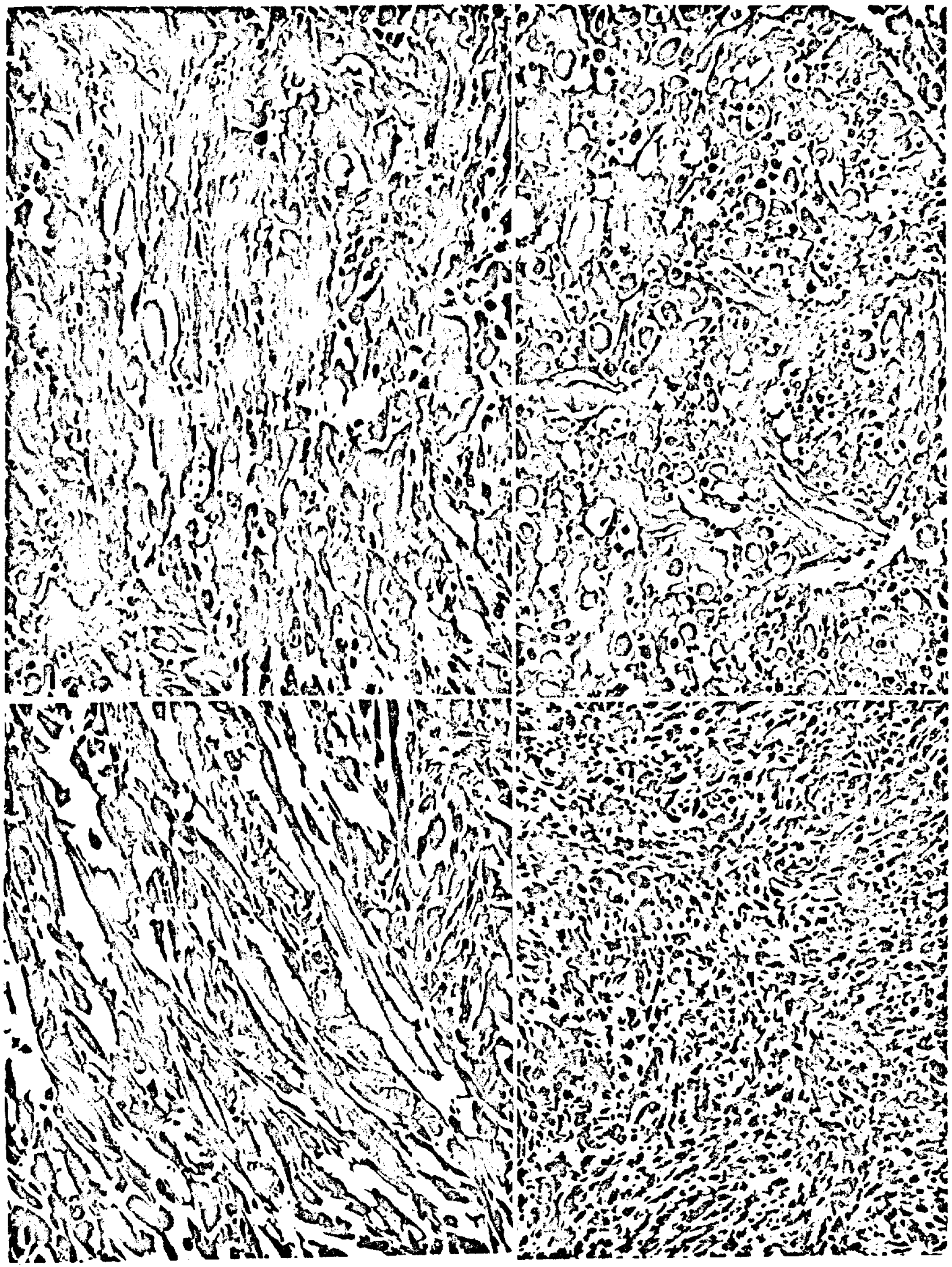

J. Nutrition
23:603-12 (1942)

THE RELATION OF THE SULPHUR AMINO ACIDS TO THE TOXICITY OF COBALT AND NICKEL IN THE RAT

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(Received for publication January 3, 1942)

Possible interrelationships of cobalt, cysteine and choline are suggested by the observations of a number of investigators. Davis ('39) noted that choline prevents cobalt polycythemia in dogs; Michaelis and co-workers (Michaelis, '29; Michaelis and Barron, '29; Michaelis and Yamaguchi, '29; Michaelis and Schubert, '30; and Schubert, '31, '33) studied the formation and structure of complexes of cysteine with cobalt and nickel; and, Beeston and Channon ('36) and Mulford and Griffith ('42) have investigated the relation of choline to cystine. In view of the above findings a study was made of the utilization of sulphur amino acids in diets containing either added cobalt or nickel, with and without supplements of choline. The data reported in this paper clearly indicate the importance of the cobalt-cysteine relationship in rats because the injurious character of cobalt-containing food mixtures is readily decreased by administrations of either methionine, cystine, or cysteine, especially the latter. No evidence was found of the participation of choline in this mechanism. The concept that cysteine may act as a detoxicating agent for metals such as cobalt has not been reported previously as far as we have been able to determine, although Launoy ('34) found that cysteine is used in the detoxication of antimony, and Shinohara ('35) suggested the formation of a thiol complex in the detoxication of mercury.

EXPERIMENTAL

Young male rats, 20 to 26 days of age and 38 to 42 gm. in weight were used in all of the experiments. Diets were fed ad libitum and food consumption was measured, but these data are not included in this paper. The basal diets contained purified casein 12 to 30%, sucrose 37 to 55, lard 19, dried brewer's yeast[1] 6, agar 2, fortified fish liver oil 1, salt mixture 4 (Hawk and Oser, '31) and calcium carbonate 1%. Supplements of choline, sulphur amino acids and metallic salts either were added to the basal diets or administered separately as noted in the tables. Solutions to be injected were made as nearly isotonic as was possible. Animals on the stock diet were used in the acute toxicity experiments and were fasted for 4 hours before oral administration of supplements.

The effect of supplementary cystine and choline on the toxicity of cobalt and other metals. Groups 7, 13 and 19 (table 1) show the effect of 0.12% of $CoSO_4 \cdot 7H_2O$ on the rate of growth of rats on diets containing 12, 18 and 30% of casein. The greater protection on the higher protein diets was probably due to the increased level of sulphur amino acids because the effect of the cobalt was partially neutralized by a supplement of 0.5% of cystine (groups 9, 15 and 20). Groups 17, 14 and 16 demonstrate the effect on growth of increasing the cobalt concentration in the same basal diet. Groups 8, 9 and 10 illustrate the failure of choline to affect the toxicity of cobalt. In these experiments the injurious level of cobalt was approximately that reported by Stare and Elvehjem ('33), Josland ('36) and Josland and McNaught ('38). The data in table 1 show without question the efficacy of the cystine supplement although group 21 was the only group which grew normally on a diet containing cobalt. As will be shown later, cystine is very much less active than cysteine in the prevention of cobalt toxicity.

Table 2 shows the results of feeding diets containing equivalent amounts of cobalt, nickel, zinc, manganese, iron and copper. Of these elements only cobalt and copper showed evi-

[1] Anheuser-Busch, Strain G.

dence of marked toxicity which was partially prevented by cystine. The poor growth on the diets containing copper may have been due in part to loss of cystine as the insoluble cuprous-cysteine complex but apparently there was loss of other nutritive value because these food mixtures deteriorated rapidly on standing. This was also true if cuprous copper was used (unpublished experiments). Deterioration of the cobalt-containing food mixtures was not observed. The stimulation of growth resulting from a supplement of cystine in the

TABLE 1

The effect of supplementary cystine and choline chloride on the rate of growth of 40-gm. male rats on diets containing toxic levels of cobalt.

GROUP [1]	DIETARY CASEIN	SUPPLEMENT			AVERAGE TOTAL GAIN IN WEIGHT			
		Cobalt [2]	Cystine	Choline chloride	5 days	15 days	30 days	50 days
	%	%	%	%	gm.	gm.	gm.	gm.
1	12	0	0	0.05	12	34	69	112
2	12	0	0	0.30	12	37	76	122
3	12	0	0.5	0.05	15	44	90	140
4	12	0	0.5	0.30	13	39	97	146
5	12	0	0.5	1.00	12	43	89	140
6	12	0.12	0	0.05	—5	—5	..	...
7	12	0.12	0	0.30	—5	—6	..	...
8	12	0.12	0.5	0.05	12	35	68	92
9	12	0.12	0.5	0.30	11	33	70	92
10	12	0.12	0.5	1.00	10	26	50	75
11	18	0	0	0.3	17	42	84	...
12	18	0	0.3	0.3	16	51	97	152
13	18	0.12	0	0.3	0	7	10	...
14	18	0.12	0.3	0.3	11	24	35	40
15	18	0.12	0.5	0.3	12	39	71	103
16	18	0.24	0.3	0.3	0	8	..	...
17	18	0.06	0.3	0.3	13	40	75	113
18	18	0.06	1.0	0.3	19	58	98	135
19	30	0.12	0	0.3	5	25	26	...
20	30	0.12	0.5	0.3	15	46	83	...
21	30	0.06	0.5	0.3	12	50	97	142

[1] Six rats per group.

[2] Expressed as per cent of cobaltous sulphate ($CoSO_4 \cdot 7H_2O$).

cystine-deficient 18% casein diet (Mulford and Griffith, '42) is also illustrated by the data in table 2.

The effect of supplementary methionine and cysteine on the toxicity of cobalt and the effect of intraperitoneal injections of the cobalt-cysteine (1:3) complex. Table 3 shows the comparative effects of cystine, cysteine and methionine on cobalt poisoning. Cysteine (groups 3 and 4) was more than twice as

TABLE 2

The effect of supplementary cystine on the rate of growth of 40-gm. male rats on diets[1] containing either added cobalt, nickel, zinc, manganese, iron or copper during a 30-day period.

GROUP[2]	SUPPLEMENT[3]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT	GROUP[2]	SUPPLEMENT[3]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT
		%	gm.			%	gm.
1	0.12% $CoSO_4 \cdot 7H_2O$	0	10	10	0.06% $MnSO_4$ (anhyd.)	0	95
2	0.12% $CoSO_4 \cdot 7H_2O$	0.3	35	11	0.06% $MnSO_4$ (anhyd.)	0.3	107
3	0.12% $CoSO_4 \cdot 7H_2O$	0.5	71	12	0.06% $MnSO_4$ (anhyd.)	0.5	107
4	0.11% $NiSO_4 \cdot 6H_2O$	0	78	13	0.11% $Fe_2(SO_4)_3$+Aq.	0	84
5	0.11% $NiSO_4 \cdot 6H_2O$	0.3	103	14	0.11% $Fe_2(SO_4)_3$+Aq.	0.3	100
6	0.11% $NiSO_4 \cdot 6H_2O$	0.5	97	15	0.11% $Fe_2(SO_4)_3$+Aq.	0.5	109
7	0.12% $ZnSO_4 \cdot 7H_2O$	0	90	16	0.11% $CuSO_4 \cdot 5H_2O$	0	30
8	0.12% $ZnSO_4 \cdot 7H_2O$	0.3	106	17	0.11% $CuSO_4 \cdot 5H_2O$	0.3	57
9	0.12% $ZnSO_4 \cdot 7H_2O$	0.5	113	18	0.11% $CuSO_4 \cdot 5H_2O$	0.5	72

[1] Basal diet: 18% casein + 0.3% choline chloride.

[2] Six rats per group.

[3] Approximately equivalent concentrations.

effective as cystine (group 2) or methionine (groups 6, 7 and 8). Group 5 shows the growth response following the oral administration of cysteine to rats after 20 days on the growth-inhibiting cobalt diet. The complex of cobalt and cysteine, while unquestionably less toxic than the cobalt alone, may in itself retain some toxicity. Lethal quantities of cobalt were injected intraperitoneally as the cobalt-cysteine complex (1:3) with partial inhibition of growth as the only apparent effect (groups 9 and 10). In these groups it was not evident whether the inhibition of growth was due to the complex itself or whether some dissociation of the complex in the body liberated the much more toxic cobalt. The beneficial effect of supplements of cystine in the diets of rats receiving injections of the complex supported the latter suggestion (groups 11 and 12).

TABLE 3

The effect of the administration of cobalt with either cystine, cysteine or methionine on the rate of growth of 40-gm. male rats on diets containg 18% casein + 0.3% choline chloride.

GROUP [1]	SUPPLEMENT	ADDED COBALT [2]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT 20 days	30 days
		%	%	gm.	gm.
1		0	0.3	70	97
2		0.12	0.3	30	35
3	0.39% cysteine hydrochloride	0.12	0	61	87
4	0.195% cysteine hydrochloride	0.12	0	41	58
5	20 mg. cysteine hydrochloride per os daily after 20th day	0.12	0	—4	17
6	0.31% dl-methionine	0.12	0	30	..
7	0.62% dl-methionine	0.12	0	44	..
8	1.24% dl-methionine	0.12	0	51	..
9 [3]	6.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0	60	83
10 [3]	19.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0	26	45
11 [3]	6.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0.5	75	103
12 [3]	19.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0.5	46	67

[1] Six rats per group.
[2] Expressed as per cent of cobaltous sulphate ($CoSO_4 \cdot 7H_2O$).
[3] Daily intraperitoneal injection of 1 or 3 cc. of a brown solution containing 1.7 mg. of cysteine hydrochloride and 0.9 mg. of $NaHCO_3$ per 1 mg. of $CoSO_4 \cdot 7H_2O$.

Toxicity and detoxication of cobalt by cysteine, glutathione (GSH), and methionine. High mortality resulted within 24 hours after the oral administration of 40 mg. and the intraperitoneal administration of 9 mg. of cobaltous sulphate (table 4, groups 3 and 7). The intraperitoneal injection of cysteine was effective in permitting survival following either method of administration of the cobalt (groups 4 and 8). Furthermore, the cobalt-cysteine complex (1:3) was without lethal effect except at high levels (groups 5, 9 and 10). The complex was prepared as described by Schubert ('31) and contained 20 mg. of $CoSO_4 \cdot 7H_2O$, 34 mg. of cysteine hydrochloride and 17 mg. of $NaHCO_3$ per 3 cc. of solution. The unneutralized cobalt-cysteine complex gave a deep red solution which became brown after neutralization. On standing this solution darkened and deposited a black precipitate. Similar results followed the administration of either the red or brown solution. The solution in the peritoneal cavity was brown within 15 minutes after the separate injections of cobalt and of unneutralized cysteine hydrochloride. The injection of the brown complex was followed by its rapid absorption from the peritoneal cavity as was evidenced by the darkening of the urine, eyes, paws and subcutaneous tissues within 5 minutes after the administration. Reduced glutathione gave the same colored solutions with cobalt as did cysteine and the mixture of cobalt and glutathione was non-toxic (group 11).

Groups 12 to 16 (table 4) show the partial protection afforded by methionine against the toxicity of cobalt. The effect was less marked than that of cysteine, as was also the case in the growth experiments (table 3). It is not possible from the present data to determine whether methionine was effective either because of its conversion to cysteine or because of the formation of a cobalt-homocysteine complex.

The effect of cysteine on the toxicity of nickel. Michaelis and Barron ('29) have reported the similar behaviour of nickel and cobalt with respect to the formation of complexes with cysteine. Groups 17 to 23 (table 4) show that the toxicity of nickel is also decreased by the administration of cysteine. In agreement with Caujolle ('39) nickel appeared more toxic than cobalt insofar as mortality from single administration was concerned. However, as noted in table 2, nickel added directly to the ration proved much less toxic than an equivalent level of cobalt. Further work is needed to clarify these apparently contradictory results.

TABLE 4

The effect of cysteine[1] and of dl-methionine on the toxicity of cobalt and the effect of cysteine[1] on the toxicity of nickel in 40-gm. male rats.

GROUP	COBALT[2] USED	NICKEL[3] USED	PROCEDURE	NO. OF RATS	24 HOUR MOR-TALITY
	mg.	*mg.*			%
1	16	0	Per os	9	0
2	30	0	Per os	9	33
3	40	0	Per os	17	71
4	40	0	Cobalt per os + intraperitoneal injection of 34 mg. cysteine	16	0
5[4]	40	0	Cobalt-cysteine complex per os	21	0
6	6	0	Intraperitoneal injection	31	58
7	9	0	Intraperitoneal injection	27	89
8	16	0	Intraperitoneal injection of cobalt and 27 mg. cysteine	12	0
9[4]	20	0	Intraperitoneal injection of cobalt-cysteine complex	21	0
10[4]	40	0	Intraperitoneal injection of cobalt-cysteine complex	15	67
11[5]	20	0	Intraperitoneal injection of cobalt-glutathione complex	5	0
12	40	0	Cobalt and 64 mg. methionine per os	10	0
13	10	0	Intraperitoneal injection of cobalt + 64 mg. methionine per os	17	53
14	10	0	Intraperitoneal injection of cobalt + 26 mg. of methionine after 3 hourly injections of 12 mg. methionine	20	25
15	10	0	Two intraperitoneal injections of 5 mg. cobalt 5 hours apart + 32 mg. methionine per os one-half hour before and after first cobalt injection	14	0
16	10	0	Two intraperitoneal injections of 5 mg. cobalt 5 hours apart	5	100
17	0	20	Per os	28	64
18	0	20	Nickel per os + intraperitoneal injection of 34 mg. cysteine hydrochloride	12	50
19	0	20	Nickel per os + intraperitoneal injection of 17 mg. cysteine hydrochloride after 0 and 3½ hours	16	25
20	0	3	Intraperitoneal injection	7	14
21	0	6	Intraperitoneal injection	10	90
22	0	10	Intraperitoneal injection	6	86
23[6]	0	10	Intraperitoneal injection of nickel-cysteine complex	10	0

[1] Cysteine hydrochloride and one equivalent of $NaHCO_3$ were used.

[2] Expressed as mg. of $CoSO_4 \cdot 7H_2O$.

[3] Expressed as mg. of $NiSO_4 \cdot 6H_2O$.

[4] Brown solution of cobalt-cysteine (1:3) complex containing 1.7 mg. cysteine hydrochloride and 0.9 mg. $NaHCO_3$ per mg. of $CoSO_4 \cdot 7H_2O$.

[5] Brown solution of cobalt-glutathione (1:3) complex containing 3.33 mg. glutathione (GSH) and 1.5 mg. $NaHCO_3$ per mg. of $CoSO_4 \cdot 7H_2O$.

[6] Nickel-cysteine (1:3) complex containing 1.7 mg. of cysteine hydrochloride and 0.9 mg. of $NaHCO_3$ per 1 mg. of $NiSO_4 \cdot 6H_2O$.

DISCUSSION

The above data suggest that the toxic effects of cobalt and nickel and possibly of copper may be largely, if not completely, neutralized by the presence in the diet of sufficient sulfhydryl compounds, such as, cysteine, reduced glutathione, or homocysteine (from methionine). These compounds are believed to detoxicate cobalt by the formation of less toxic complexes of the type described by Michaelis in the case of cobalt and cysteine. It may be that the principal effect in cobalt poisoning is that which results from the binding in complex formation of sulfhydryl compounds in the tissues. Indeed, it is conceivable that interference with cellular oxidations due to formation of such complexes, with glutathione for instance, may be the stimulus to the hematopoietic system which causes so-called cobalt polycythemia. Furthermore, these data suggest that consideration be given the possibility that dietary supplements of sulfhydryl compounds, or their precursors, may alter the outcome of studies of cobalt metabolism, and that dietary supplements of cobalt may alter the outcome of studies of sulfhydryl metabolism. In addition, the complex of cobalt and the sulfhydryl compound may itself be physiologically active.

It is significant that Barron and Barron ('36) and Davis ('40) have reported studies on the relation of ascorbic acid to cobalt polycythemia and have concluded that cobalt may bring about erythropoiesis by interference with some respiratory function of this vitamin. Although preliminary unpublished experiments have failed to demonstrate any influence of ascorbic acid on the detoxication of cobalt by cysteine in the rat, an interrelationship may exist between cobalt, ascorbic acid and a sulfhydryl compound, such as, glutathione. It is not possible at this time to coordinate the results of the present paper with the recognized role of cobalt as a dietary essential required for the prevention of anemia. The latter studies and the associated effects of an unisolated organic substance in liver extracts have been reviewed by Underwood ('40).

SUMMARY

1. The marked inhibition of growth of young rats due to 0.12% of cobaltous sulphate in an 18% casein diet is largely prevented by supplements of methionine, cystine or cysteine, especially the latter.

2. The high mortality due to either the oral or intraperitoneal administration of very toxic levels of either cobalt or nickel is prevented by the simultaneous and separate administration of cysteine.

3. The complex of cobalt and cysteine formed in vitro is relatively non-toxic.

4. Cobalt poisoning may be due to the fixation and loss of sulfhydryl compounds in tissues with resulting interference with oxidative mechanisms.

We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

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Arch Envir. Health
14(4) 604-613(1967)

Acute Pathological Reactions to Administration of Nickel Carbonyl

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EPIDEMIOLOGICAL[1-9] and clinical[10-14] investigations have implicated nickel as an etiologic factor in cancers of the respiratory tract. Increased incidence of respiratory cancer has been found among nickel workers in Great Britain,[6] Germany,[15] Norway,[11] Japan,[16] Russia,[17,18] and Canada.[19] For example, Doll[20,21] has reported that during the years from 1938 to 1956, 35.5% of the deaths among nickel workers in Glamorganshire, Wales were caused by cancers of the lung and nasal passages. The incidence of pulmonary cancer among these nickel workers was nine times greater than was observed among other industrial workers in the same region.[20,21] Nickel has been implicated as a carcinogen in tobacco smoke[22,23] and in the atmospheres of industrial communities.[24]

The carcinogenic properties of nickel compounds have been established by numerous experimental studies.[25-46] As summarized in Table 1, administration of nickel compounds by a variety of routes has induced malignant tumors in rats, guinea pigs, and rabbits. Payne[43,44] has demonstrated an inverse correlation between the carcinogenicity of nickel compounds and their solubility in aqueous media. Nickel dust, nickel sulfide (Ni_3S_2), nickel carbonate ($NiCO_3$), nickel hydroxide ($Ni[OH]_2 \cdot H_2O$), and nickel oxide (NiO) all have solubilities of less than 25 $\mu g/ml$ in saline at 37 C.[44] Osteogenic sarcomas, fibrosarcomas, and rhabdomyosarcomas have been induced in experimental animals by injections of insoluble nickel compounds suspended in lanolin, gelatin, penicillin, fowl serum, and sheep fat, and by implantations of nickel pellets, discs, and chips. Implantation of nickel in the lungs and pleural cavities of rats has resulted in the development of sarcomas, but has not induced pulmonary carcinomas of the histological types which are observed among nickel workers. The experimental induction of such carcinomas by nickel has only been accomplished following exposures by inhalation. Hueper[19,37] has reported an anaplastic pulmonary carcinoma in a guinea pig exposed to inhalation of powdered metallic nickel, and Sunderman and coworkers[15,46] have reported pulmonary carcinomas in six rats exposed to inhalation of nickel carbonyl ($Ni[CO]_4$). Two of these tumors were squamous cell carcinomas, two were adenocarcinomas, and two were anaplastic carcinomas.

From a methodological viewpoint, exposure of rats to inhalation of nickel dust poses difficulty in controlling particle size and achieving uniform distribution and retention in the lungs. These disadvantages do not pertain to the inhalation of the vapors of nickel carbonyl, a volatile liquid which boils at 43 C. However, the extreme toxicity of nickel carbonyl* has necessitated the use of

Submitted for publication June 15, 1966; accepted Sept 14, 1966.

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Read before the American Society of Pathologists and Bacteriologists, Cleveland, March 4, 1966.

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*The American Conference of Governmental Hygienists[47] has placed the threshold limit value for nickel carbonyl in industrial atmospheres at one part per billion (7 μg/cu m). By contrast, the threshold limit value for hydrogen cyanide is ten parts per million (11 mg/cu m), and the value for carbon monoxide is 100 parts per million (110 mg/cu m).

Table 1.—*Nickel Carcinogenesis in Experimental Animals*

Author	Date	Species	Compound	Route	Malignant Tumors
Hueper[35]	1952	Rat	Ni dust in lanolin	Intrafemoral intrapleural	Sarcomas
Hueper[36]	1955	Rat and rabbit	Ni dust in gelatin	Intrafemoral subcutaneous	"
Hueper[19,37]	1958	Guinea pig	Ni dust	Inhalation	Anaplastic carcinoma
Sunderman et al[45]	1959	Rat	$Ni(CO)_4$ vapor	"	Squamous cell and adenocarcinomas
Mitchell et al[41]	1960	"	Ni pellets	Subcutaneous	Sarcomas
Hueper and Payne[39]	1962	"	Ni dust in gelatin	Intrapulmonary	"
Gilman[27]	1962	"	Ni_3S_2; NiO dusts in penicillin	Intramuscular	"
Gilman et al[25,28-30,34]	1963-1966	"	Ni_3S_2 dust, discs, chips	"	"
Jasmin[40]	1963	"	Ni_3S_2 dust in pencillin	"	"
Noble and Capstick[42]	1963	"	Ni_3S_2 dust	"	"
Heath and Daniel[33]	1964	"	Ni dust in fowl serum	"	"
Payne[43,44]	1964, 1965	"	Ni_3S_2; $NiCO_3$; NiO dusts in sheep fat	"	"
Sunderman and Donnelly[46]	1965	"	$Ni(CO)_4$ vapor	Inhalation	Squamous cell, anaplastic and adenocarcinomas

special exposure chambers[17] and detection devices[48,49] to safeguard investigators engaged in inhalation experiments. During the past year, our laboratory has investigated alternative techniques of introducing nickel into the lungs in an attempt to develop a more convenient and less hazardous method for the induction of pulmonary carcinomas. These investigations have revealed that administration of nickel carbonyl parenterally to rats provokes acute clinical and pathological reactions which closely resemble those which have previously been observed following inhalation. In contrast to exposure by inhalation, administration of nickel carbonyl by parenteral routes has the advantages of safety, simplicity, and quantitative dosage.

Methods

The experimental animals were male rats of the Sprague-Dawley strain weighing from 100 to 125 gm. All manipulations employing nickel carbonyl were performed in a fume hood. Nickel carbonyl was dispensed from a small lecture cylinder into a 1 ml glass-stoppered tube. Microsyringes with capacities of 5μl to 25μl were employed for injections parenterally by intravenous, subcutaneous, and intraperitoneal routes. Injections were made intravenously into a lateral vein at the base of the tail, following dilatation of the vein by exposure to a heat lamp. Injections were made subcutaneously in the interscapular region.

In order to determine median lethal dose (LD_{50}) values, nickel carbonyl was administered by each of the parenteral routes to groups of 40 rats. Each group was divided into five subgroups of eight rats, corresponding to the following dosage levels: 0.57, 1.13, 2.26, 3.39, and 4.52 mg of nickel per 100 gm. These dosage levels are equivalent to the following volumes of nickel carbonyl: 1.25, 2.5, 5.0, 7.5, and 10μl/100 gm. Except for being weighed each morning, the rats were allowed to remain undisturbed in their cages. Rats which died in the course of the LD_{50} experiments were autopsied, and tissues were examined histologically. One month after the injections of nickel carbonyl, the experiments were terminated and the surviving rats were killed, Computations of the LD_{50} levels were performed by the probit method of Miller and Tainter.[51]

In order to follow the development of the acute pathological reactions, nickel carbonyl was administered intravenously in LD_{50} doses to a group of 75 rats. Fifteen rats which received sham injections served as controls. The rats were killed by an injection of pentobarbital intracerebrally at the following intervals after injection: 0, 1, 4, 8, and 24 hours, and 2, 4, 7, 14, and 21 days. The following tissues were examined histologically after fixation in Bouin's fluid: thymus, heart, lungs, liver, pancreas, intestines, spleen, adrenals, kidneys, bladder, testes, and tail. The right lung was fixed in the distended state by bronchial infusion of Bouin's fluid. The left lung was fixed in the collapsed state. The tissues were sectioned at 5μ and were stained by the following procedures: hematoxylin and eosin, periodic acid-Schiff (PAS), phosphotungstic acid-hematoxylin, Masson's trichrome, and Wilder's method for reticulum. Portions of lungs from selected rats were fixed in osmium tetroxide, embedded in epoxy plastic, and sectioned at 0.5μ to 2.5μ. These sections were examined by phase microscopy and were also stained with toluidine blue.

Results

Clinical Observations.—*Initial Reactions.*—The initial reactions to nickel carbonyl administered parenterally resembled those previously reported following inhalation.[49,52-55] The rats pawed at their snouts immediately after the injections of nickel carbonyl. Approximately 5% of the rats died within the first hour after administration of nickel carbonyl in LD_{50} dosage by the intravenous route. Some of the rats which received nickel carbonyl in a lateral tail vein intravenously developed transient paralysis of the ipsilateral hind leg. Weakness, anorexia, diminished intake of water, and loss of weight were observed in all of the rats during the first 24 hours after injection.

Delayed Reactions.—The rats usually recovered from the initial reactions by the end of the first day and appeared essentially normal on the second day. Delayed reactions generally developed on the third day after injection of nickel carbonyl in LD_{50} dosages by each of the parenteral routes. The delayed manifestations resembled the reactions which were previously reported following inhalation of nickel carbonyl in man[55-65] and experimental animals.[49,52-54] These reactions included tachypnea and dyspnea, profound weakness, loss of weight, inactivity, irritability, and ruffled fur. On the third day, many of the rats which received nickel carbonyl by injection intraperitoneally developed abdominal distention which became most severe on the fourth and fifth days. In all of the groups, death usually occurred on the fourth and fifth days following injection. During the hours preceding death, the rats developed extreme dyspnea, with cyanosis of the oral mucosa. The rats which survived began to regain weight on the sixth or seventh days. No fatalities occurred more than eight days following injections of nickel carbonyl.

Mortality Statistics.—*LD_{50} Values.*—The LD_{50} values for nickel carbonyl administered by parenteral routes are listed in Table 2 and are contrasted with previously determined LD_{50} values for exposure by inhalation. The LD_{50} for intravenous injection was 2.2 mg of Ni per 100 gm of body weight, with a standard error (SE) of ± 0.11. The LD_{50} dosage by subcutaneous injection (2.1 mg Ni/100 gm) did not differ significantly from that by intravenous injection. It may be noted from the SE, ± 0.42, that the mortality response following injection of nickel carbonyl subcutaneously was more variable than was obtained by intravenous injection. The LD_{50} dosage by injection intraperitoneally was 1.3 ± 0.14 mg of Ni per 100 gm. As will be discussed subsequently, the greater toxicity of nickel carbonyl following injection intraperitoneally is attributable to peritonitis. As shown in Table 2, the LD_{50} of nickel carbonyl was greater by parenteral

Table 2.—*Acute Toxicity of Nickel Carbonyl in Rats*

Authors	Route	LD_{50} ± SE (mg Ni/100 gm)	
Present study	Intravenous	2.2 ± 0.11	
" "	Subcutaneous	2.1 ± 0.42	
" "	Intraperitoneal	1.3 ± 0.14	
		(mg Ni/liter of air)	(mg Ni/rat*)
Barnes and Denz[53]	Inhalation	0.14 (30 min)	0.17
Kincaid et al[54]	"	0.08 (30 min)	0.10
Sunderman and Hackett†	"	0.20 (15 min)	0.12

*Assuming ventilation volume of 40 ml/min.[23]
†Unpublished data.

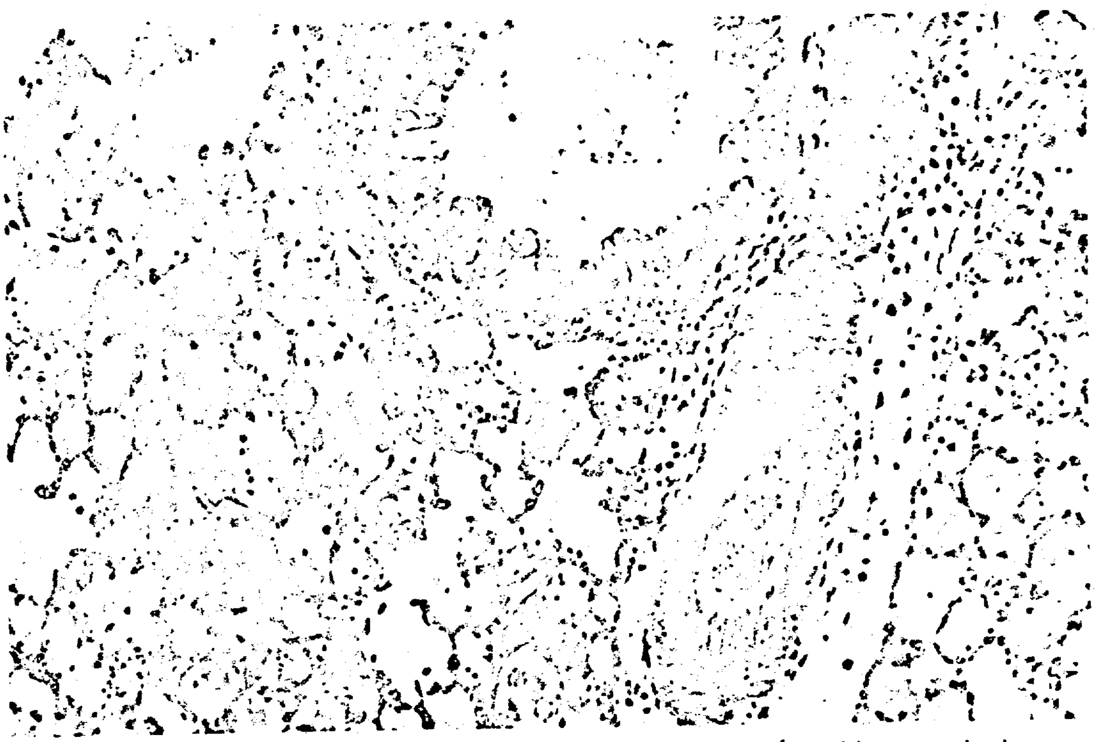


Fig 1.—Lung from a rat sacrificed four days after injection of nickel carbonyl intravenously, demonstrating perivascular and intraalveolar edema, and thickening of the alveolar walls (PAS, × 100).

routes than by inhalation. Assuming a ventilation volume for the 100 gm rat of 40 ml/minute,[23] and assuming complete retention of nickel, the LD_{50} of inhaled nickel carbonyl is approximately 0.12 mg of Ni per 100 gm.

Pathological Observations.—*Gross Findings Following Administration of $Ni(CO)_4$ Intravenously.*—No gross pathological changes were observed during the first 24 hours after injection of nickel carbonyl intravenously. On the second day following injection, generalized visceral engorgement was noted, particularly affecting the lungs and liver. Pulmonary congestion reached maximal severity on the fourth and fifth days. The lungs of all rats which were killed on the fourth day were dark red, firm, and distended. The cut surfaces of the lungs were slightly bulging, airless, and exuded serosanguinous fluid. Gray areas of subpleural pulmonary consolidation were present in many of the rats killed on the fourth day. On the second to fourth days, the liver showed moderate central congestion. By the

Fig 2.—Lung from a rat killed four days after injection of nickel carbonyl intravenously, demonstrating hypertrophy and proliferation of alveolar lining cells (arrow [PAS, × 400]).

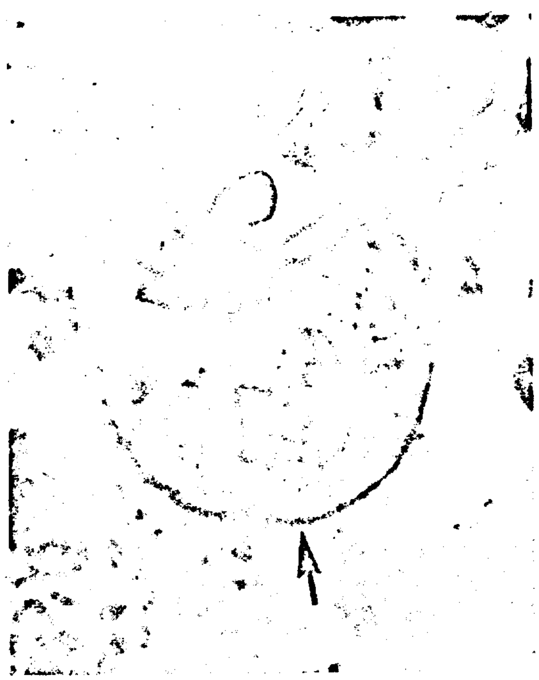


Fig 3.—Lung from a rat killed four days after injection of nickel carbonyl intravenously, demonstrating atypical mitosis (arrow [PAS, × 2,500]).

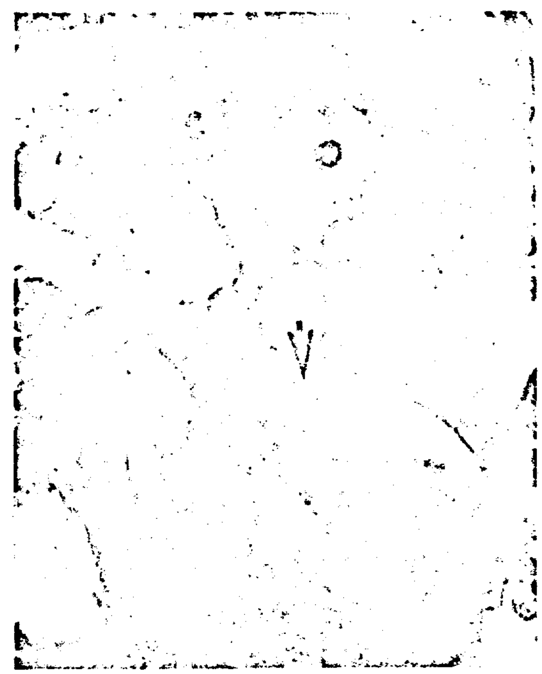
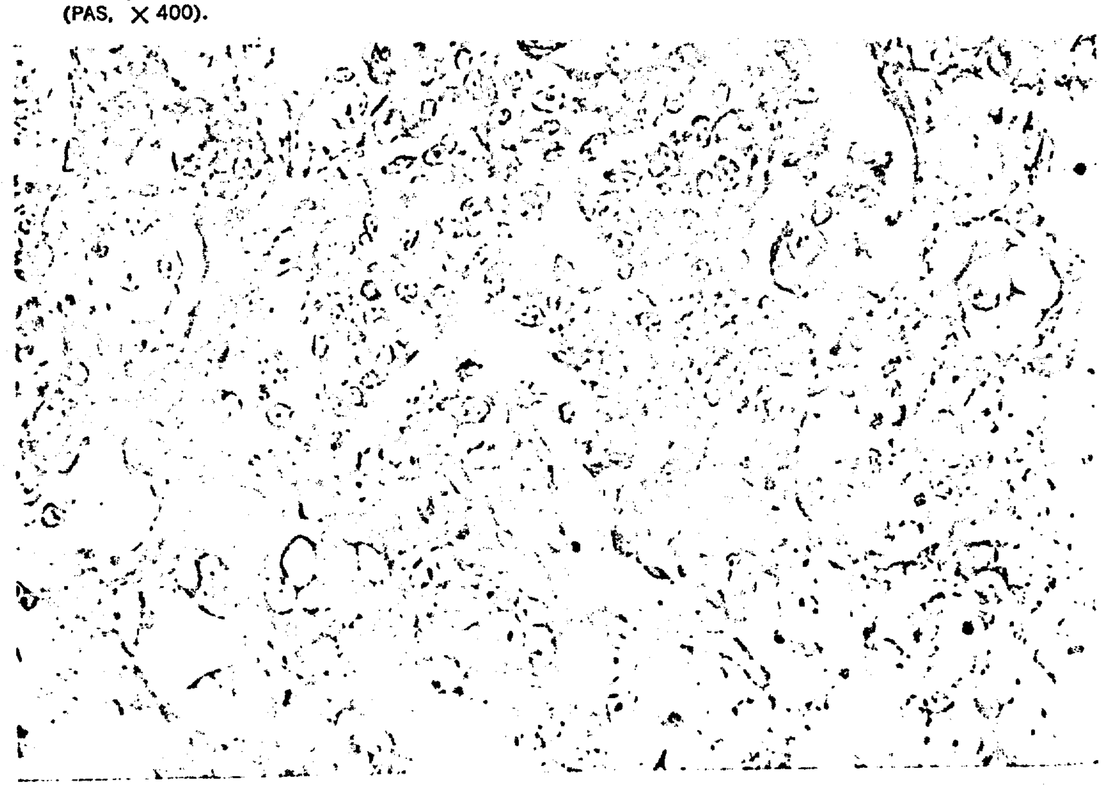


Fig 4.—Lung from a rat killed four days after injection of nickel carbonyl intravenously, demonstrating adenomatoid hyperplasia (arrow [PAS, × 400]).

Fig 5.—Lung from a rat killed four days after injection of nickel carbonyl intravenously, demonstrating an area of alveolar destruction, with adenomatous hyperplasia and fibroblastic proliferation (PAS, × 400).



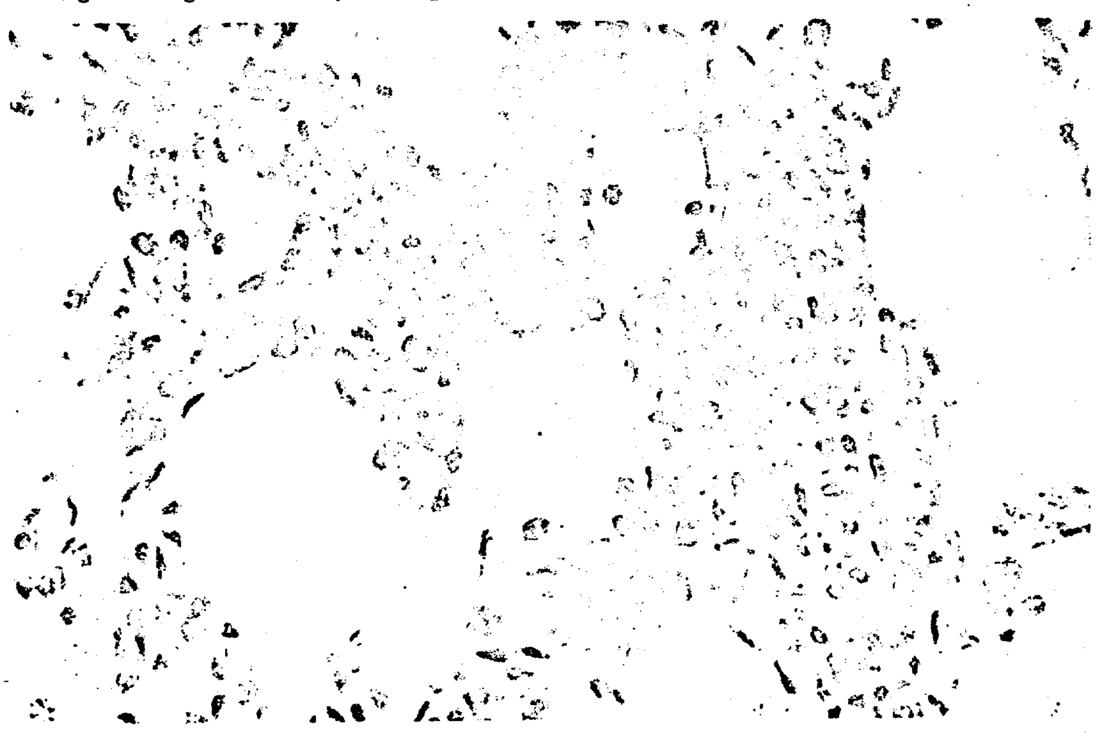
seventh day, the gross pathological changes in the lungs and liver had diminished, and, by the end of the second week the organs were grossly normal, except for focal pulmonary consolidation.

Microscopic Findings Following Administration of $Ni(CO)_4$ Intravenously.—The most noteworthy pathological changes were present in the lungs. By one hour after injection, perivascular edema developed around the larger pulmonary vessels. Four hours after injection the perivascular edema was increased, and polymorphonuclear leukocytes were present in the vessels and perivascular tissues. At 24 and 48 hours, the outer layers of the vascular media were severely edematous. Polymorphonuclear leukocytes accumulated in the capillaries and, to a lesser degree, within the alveolar spaces. The alveolar lining cells were numerous and were increased in size. The lining epithelium of the bronchioles was taller than normal, with enlarged nuclei and with increased number of mitoses.

The pathological reactions in the lungs were maximal on the fourth day after injection of nickel carbonyl (Fig 1). The most striking cellular alterations affected the alveolar lining cells, which were extremely hypertrophied and hyperplastic (Fig 2). Their nuclei were enlarged, and their nuclear membranes were irregular and stained darkly. Dense nucleoli were present, usually adjacent to the nuclear membrane, and surrounded by clear nucleoplasm. Binucleated cells were common, and numerous atypical mitoses were noted (Fig 3). The majority of the alveolar lining cells had abundant cytoplasm. Tangled fibrillary networks, which stained purple by the hematoxylin and eosin and PAS techniques, were present throughout the cytoplasm. The interstices of the fibrillary networks were clear, giving the cytoplasm a reticulated appearance. Because of the distortions of the alveolar lining cells, no differentiation could be made between type 1 and type 2 pneumocytes.

Foci of adenomatous transformation were observed in the lungs of the rats which were killed on the fourth day after administration of nickel carbonyl (Fig 4). Within these adenomatous foci, the alveoli were lined with cuboidal or columnar cells without cilia. The round nuclei of these cells were

Fig 6.—Lung from a rat killed 21 days after injection of nickel carbonyl intravenously, demonstrating thickening of alveolar septae owing to interstitial hypercellularity (PAS, × 250).



basal or central in location, and contained prominent nucleoli. The cytoplasm of these cells was amorphous and stained lightly by all techniques. These cells resembled lining cells of terminal bronchioles, but only rarely were there any apparent connections between these cells and the terminal bronchioles.

Microscopic study of the areas of the lungs which appeared consolidated on gross examination revealed destruction of the alveoli (Fig 5), extensive fibroblastic proliferation, and accumulation of mononuclear histiocytes. Large mononuclear cells were abundant in the interstitial spaces throughout the lungs, as well as within the alveolar spaces. The cytoplasm of these mononuclear cells was frequently vacuolated. The bronchiolar epithelial cells were increased in height, and contained increased amounts of PAS-positive material within their cytoplasm. The endothelial cells of small and medium-sized vessels were characterized by prominent nucleoli and abundant, eosinophilic cytoplasm. Staining by the PAS technique revealed diffuse thickening of the alveolar basement membrane. Throughout the lungs the basement membrane was changed from its normal thin and delicate structure to an uneven, fibrillary appearance.

Seven days after injection of nickel carbonyl, the pathological reactions in the lungs decreased in intensity. Hypertrophy and hyperplasia of the alveolar lining cells persisted, but the nucleolar and chromatin abnormalities were less prominent, and the number of mitoses was reduced. Foci of adenomatous transformation and areas of pulmonary fibrosis were apparent. Further regression of the lesions was noted at 14 days, and 21 days after injection of nickel carbonyl, microscopic examinations showed that the lungs were essentially normal, except for interstitial fibrosis (Fig 6). It should be noted that no pathological lesions were observed in the lungs of any of the control rats.

Mild pathological changes developed in the livers, kidneys, and adrenal glands of rats which had received injections of nickel carbonyl intravenously. On the fourth day, centrilobular congestion was present in the liver, associated with the loss of PAS staining. In the kidneys, sporadic vacuolization was found in the proximal convoluted tubules, but tubular necrosis was not observed. In the adrenal glands, there was intense congestion of the cortical sinusoids, but hemorrhage was absent. Sections through the injection site in the tail revealed subcutaneous edema in some animals, with venous engorgement and occasional venous thrombosis.

Pathological Findings Following Administration of $NiCO_4$ Subcutaneously and Intraperitoneally.—The gross and microscopic alterations which were observed after injections of nickel carbonyl subcutaneously and intraperitoneally were more variable in degree than those which developed after injection intravenously. The pulmonary findings in rats which received nickel carbonyl by these routes of administration were identical in character to those which were observed following injection of nickel carbonyl intravenously. The rats which received nickel carbonyl by injections intraperitoneally developed peritoneal effusions within 24 hours. Purulent peritoneal exudates were frequently observed in rats which died on the fourth to seventh days after injection intraperitoneally. Evidence of peritonitis was absent by the third week after administration of nickel carbonyl intraperitoneally.

Comment

The pulmonary parenchyma has been found to be the target tissue for nickel carbonyl, regardless of its route of administration. Injections of nickel carbonyl given intravenously have proved to be an effective method of exposing the lungs to nickel. This method of exposure to nickel is simple, rapid, and inexpensive. It appears to be more quantitative than exposures to the inhalation of nickel dust, and is less hazardous than exposure to the inhalation of nickel carbonyl. Moreover, administration of nickel carbonyl intravenously assures contact of nickel with the pulmonary parenchymal cells, bypassing the protective effects of the mucus stream and the ciliated cells. For these reasons, injections of nickel carbonyl intravenously are currently being employed in our laboratory in an attempt to induce pulmonary cancers.

The present study has demonstrated that the clinical and pathological reactions to nickel carbonyl given parenterally closely resemble the reactions which have been observed previously in man and experimental animals following the inhalation of nickel carbonyl vapor. The cytologic abnormalities which developed in the alveolar lining cells were virtually identical to those described by Barnes and Denz,[53] Brandes,[58] and by Sunderman and coworkers.[55] The peak mortality following parenteral administration of nickel carbonyl occurred approximately one to two days later than the peak mortality which was previously reported following inhalation of nickel carbonyl.[53,55] This finding is probably attributable to the fact that the previous investigators employed greater than LD_{50} doses. It should be emphasized that from a quantitative viewpoint, the toxic doses of nickel carbonyl by parenteral routes are not directly comparable to the toxic doses following exposures by inhalation. It cannot be discerned from the present investigation whether the proliferative alveolar response to administration of nickel carbonyl parenterally is a direct result of intracellular reactions of nickel, or whether it is a secondary response to destruction of the alveolar septae. A direct intracellular toxic reaction is suggested by the bizarre nuclear configurations and atypical mitoses. Alternatively, the fact that the pathological lesions did not reach maximal severity until four days after injection of nickel carbonyl suggests that the lesions may be manifestations of a reparative process. It should be emphasized that the pathological reactions in the lungs are not specific for nickel poisoning. Similar proliferation and hypertrophy of alveolar lining cells have been reported by Tennant et al[66] in the lungs of a man who died from acute mercury pneumonitis, and by Totten and Moran[67] in the lungs of rabbits treated with cortisone prior to injection of nitric acid intratracheally. The possible relationship between the acute pulmonary response to toxic agents and the mechanisms of chemical carcinogenesis in the lungs is a subject for speculation. Meyer and Liebow[68] have recently reviewed the evidence for a relationship of atypical epithelial proliferation to the development of pulmonary cancer. They have concluded that the toxic action of diverse agents upon the lung is manifested by a final common pathway which includes regenerative hyperplasia, atypical epithelial proliferation, and ultimately, neoplasia.

Armit[52] has reported that the addition of nickel carbonyl to plasma or whole blood in vitro results in the formation of a colloidal suspension of hydrated nickel carbonate, accompanied by liberation of carbon monoxide. If this reaction occurs intravascularly following the injection of nickel carbonyl by intravenous route, the observed pathological reactions may result from retention of colloidal nickel carbonate in the pulmonary capillary bed. On the other hand, the studies of Amor,[56] Ghiringhelli and Agamennone,[69] Henriot and Richet,[70] Langlois,[71] Trout,[72] and Vahlen[73] have indicated that nickel does not undergo rapid decomposition upon contact with blood or body tissues in vivo. It is possible that nickel carbonyl vapor may be excreted by the lungs following administration by parenteral routes, and that the acute pathological reactions may result from contact of the pulmonary epithelium with intact molecules of nickel carbonyl. Investigations employing $Ni^{63}(CO)_4$ are currently being undertaken in our laboratory in an attempt to settle this controversy and to elucidate the biochemical and ultrastructural alterations which are responsible for the acute toxicity of nickel carbonyl.

Summary

In previous investigations, pulmonary carcinomas have been induced in rats following inhalation of the toxic vapors of nickel carbonyl. In the present investigation, administration of nickel carbonyl parenterally has been studied in an attempt to develop a more convenient and less hazardous method of introducing nickel into the lungs. The LD_{50} values for nickel carbonyl were found to be 2.2 ± 0.11 mg Ni/100 gm, by injection intravenously; 2.1 ± 0.42 mg Ni/100 gm by injection subcutaneously; and 1.3 ± 0.14 mg Ni/100 gm by injection intraperitoneally. The acute clinical and pathological reactions to injections of nickel carbonyl by parenteral routes developed primarily in the lungs, and to a lesser degree in the liver, resembling the reactions which

were previously observed following inhalation of nickel carbonyl. Pulmonary histological changes were characterized by focal adenomatous transformation and by diffuse hypertrophy and hyperplasia of alveolar lining cells, with increased mitotic activity and cytologic abnormalities. The pulmonary parenchyma was found to be the target tissue for nickel carbonyl, regardless of its route of administration.

This investigation was supported by US Atomic Energy Commission Grant No. AT-(40-1)-3461; by American Cancer Society Grant No. E-374 A, and by National Institute of Health Grant No. FR-05361-04.

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ECOLOGY

The new science of ecology has shown that habitat is primary in determining the life of every organism, and man is no exception.—Entwistle, Clive: Roads to Ruin, *The New York Times Book Review*, Sept 4, 1966, p 3.

Journal of Biol. Chem.
112:175 (1935)

ACTIVATION OF ENZYMES

III. THE RÔLE OF METAL IONS IN THE ACTIVATION OF ARGINASE. THE HYDROLYSIS OF ARGININE INDUCED BY CERTAIN METAL IONS WITH UREASE

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(Received for publication, August 22, 1935)

Earlier papers from this laboratory have dealt with the activation of urease (1) and of papain (2), with emphasis, particularly, upon reversible inactivations by oxidizing agents and by certain organometallic compounds. This communication describes an investigation of the markedly differing activation chemistry of the enzyme, arginase. There is stressed the conspicuous rôle apparently played by metal ions in the arginase-arginine reaction, a study of which has disclosed the conditions under which hydrolysis of arginine may be extensive *when induced only by certain metal ions with urease.*

Although enzyme literature is replete with descriptions of the activating and inactivating effects of various reagents (*cf.* (3)), there have appeared only recently reports of investigations in which has been attained a measure of success in the correlation of some of these effects in terms of a rational chemistry. Progress has been impeded in part by the difficulty of obtaining most enzymes in a state of even approximate purity or in the reproduction by different workers of crude enzyme preparations possessing sufficiently constant behavior.

For our initial studies in this field we selected crystalline urease and papain, the properties of which promised relative freedom from these difficulties. Controlled inactivations of these enzymes by various oxidizing agents were found to be extensively reversible, the reversals being effected by a number of reducing substances. The inactivating effects of cuprous oxide and certain organic mercury derivatives, which are known to be mercaptide-forming, were likewise shown to be reversed by suitable reagents. The results as a whole seemed to support the tentative hypothesis that for urease or papain such effects may largely be attributed to reversible chemical actions upon thiol groups which for the present may be assumed to be integral substituents of the enzyme molecules themselves[1,2] (1, 2).

While this simple and attractive picture may well reflect a phenomenon of considerable biochemical importance, it can by no means be regarded as universally applicable. This is sufficiently illustrated by a consideration of the diverse, and often contradictory, findings of many recent investigators of arginase activation. The earlier opinions of several investigators (8, 9) concerning the rôle of organic thiol compounds, *e.g.* glutathione, as specific activators of arginase were abandoned when it was found (10) that the effects of such compounds are variable and that ferrous ion, alone or used together with sulfhydryl (11, 12) or with certain other organic compounds (13), is a much more characteristic activator. Little progress has as yet been made in defining arginase activation (14, 15) in terms of a reversible oxidation-reduction process, and there exists among various workers upon this aspect no agreement as to the significance of the results obtained.

Using a liver-enzyme preparation of satisfactory stability and potency, of a high degree of specificity[3] as arginase, and free of thiol compounds that respond to nitroprusside, we have ascertained that the arginase action neither is significantly altered by

[1] The work of Shwachman, Hellerman, and Cohen (4) disclosed a striking and perhaps significant analogy to these observations upon urease and papain in the effects of oxidation-reduction and mercaptide-forming agents upon the lytic action of hemolysin of pneumococci, Type II.

[2] The concept of enzyme activity control through reversible oxidative attack upon a grouping of the enzyme molecule was independently suggested by Bersin and Logemann (5) in a paper upon papain which appeared shortly after our communication upon urease and before our own experimental work with papain activation was completed. Maschmann and Helmert (6) suggested that the activation of cathepsins and of papain by HCN is related to the reduction to thiol of dithio groupings in the protein-enzyme molecules, and Bersin subsequently (7) interpreted his findings in terms of thiol chemistry.

[3] Unpublished work of Chester Stock and the authors.

mercaptide-forming organomercurials (*e.g.* C_6H_5HgOH) nor is enhanced by sulfhydryl compounds like cysteine. Certain other reducing agents, *e.g.* bisulfites, cyanides, and hydrogen sulfide, which are also effective papain activators, likewise fail to activate this arginase. On the other hand, we have found that arginase activity is enhanced under certain conditions by any one of the ions, Co^{++}, Ni^{++}, or Mn^{++}, as well as Fe^{++}. Indeed, the activity is apparently restored and brought to its maximum by the simple addition of cobaltous ion after treatment of the enzyme with such inhibitors as quinone, ferric ion, or iodine. Furthermore, arginase is suppressed by cyanide or by hydrogen sulfide, although partial restoration from the action of the latter is readily effected by suitable removal of the excess reagent; and the activity is more completely reestablished by the subsequent addition of an appropriate "bivalent" metal ion. These observations reveal a striking contrast between the mode of activation of arginase, on the one hand, and of urease or papain, on the other. They bring into sharp focus the probable significance of metal ion chemistry for the arginase-arginine reaction.

This is of additional interest in view of the remarkable fact, disclosed by this work for the first time, so far as we are aware, that arginine (without arginase) is readily hydrolyzed, even at pH 7.5 at 30°, in the presence of certain metal ions (Co^{++}, Ni^{++}, Mn^{++}) *when urease (crude jack bean or the crystalline protein enzyme) is also present in the reaction mixture.* Quite apart from its inherent theoretical interest, the latter discovery requires consideration in connection with certain analytical operations in addition to those actually concerned with the study of arginase catalysis.

Methods

Arginase Preparation[4]—The freshly excised liver of a calf is cut into small pieces which are placed in crushed ice, in a glass container. The chilled tissue (about 2 pounds) is ground and mixed well during 1 hour with 300 ml. of glycerol (U.S.P.). The mixture is pressed through several layers of muslin (in a meat press), yielding an extract of perhaps 50 ml. For present purposes this is discarded, and to the *residue* from this extract are added 500 ml. of water. This mixture is stirred ½ to 1 hour, after which it is

[4] *Cf.* (16).

pressed through muslin, yielding an extract of about 220 ml. The residue may be profitably reextracted and worked up further, if desired. To the *fluid extract* is added toluene, 25 ml., and, after being well shaken, the mixture is allowed to stand in a refrigerator for 12 hours. It is then centrifuged 1 to 1½ hours (at 2400 R.P.M.). The top toluene-fat layer and a small amount of solid on the bottom are discarded. To the somewhat cloudy middle layer is added acetone (U.S.P.) until the precipitation of solid material is complete. After the precipitate has settled, most of the yellow supernatant liquid is removed by decantation; the solid is collected on Buchner funnels and washed well with acetone (C.P.). It is air-dried on sheets of filter paper for removal of residual acetone, after which it is dried in a vacuum desiccator over P_2O_5. This crude enzyme powder (about 13 gm.) may be kept in a glass-stoppered bottle at room temperature. Aqueous extracts of such material provided the arginase used in this work. As implied in the foregoing, continued extraction of the liver tissue with appropriate subsequent manipulations yields more crude arginase. The details were worked out with the aid of determinations of enzyme activity upon all fractions and this guided the selection of the fraction actually used.

Arginase Solution—A solution of enzyme is freshly prepared by addition of 5 mg. of the solid preparation to each ml. of redistilled water taken; the mixture is stirred, heated at 37° for 1 hour, and filtered. The residue is discarded. The clear filtrate contains the enzyme activity and will be called hereafter *arginase solution*.

Such extracts have not yet been fully examined chemically or spectrographically. Coagulable protein is present; compounds that respond to the nitroprusside test are absent; extracts, heated with alkaline plumbite, yield PbS. It may be noted that this enzyme preparation has been found to be rather highly specific as arginase. Work by Chester Stock and the authors (to be described in a later communication) has demonstrated that this arginase fails to catalyze the hydrolysis of α-hydroxy-δ-guanidovaleric acid (argininic acid) or δ-guanidovaleric acid under conditions which permit the extensive hydrolysis of arginine.

Determination of Arginase Activity—The development of a quantitative method suited to this problem was aided by reference to the work of Jansen (17), Edlbacher (18), and Hunter (19) and their collaborators. The urea formed by hydrolysis of arginine was determined essentially by Marshall's method (20) as adapted to precision work by Van Slyke and Cullen (21). There follows a description of a *typical experiment*.

Water redistilled in glass apparatus was used throughout. Arginase solution was prepared from some arginase powder which had stood for several months. To 2 ml. of this, contained in a 25 ml. volumetric flask, were added 8 ml. of water and 5 ml. of 1 M phosphate buffer, pH 7.5. In a parallel determination there then were added 3 mg. of cobaltous chloride ($CoCl_2 \cdot 6H_2O$). Each mixture stood 15 minutes (at 25°), after which it was brought to 37° and treated with a few drops of caprylic alcohol and a solution of 45 mg. of arginine carbonate (or an equimolar amount of arginine hydrochloride) in 1 ml. of water. Hydrolysis was permitted to proceed at 37° for exactly 2 hours; the flask was plunged into boiling water and held there for 10 minutes, after which it was cooled to room temperature and the following additions made, in the order named: phosphate buffer (1 M, pH 7.5) 5 ml., sodium bisulfite 5 mg., sodium cyanide 16 mg., and 0.1 gm. of jack bean urease powder (22). The volume was brought to 25 ml., a few drops of caprylic alcohol added, and the mixture kept at 30° overnight, after which it was filtered. Of the aqueous filtrate, 10 ml. were added to 10 ml. of saturated K_2CO_3 solution and a few drops of caprylic alcohol, and the ammonia carried into 0.02 N HCl by aeration for 2 hours. The excess HCl was titrated with 0.02 N NaOH, chlor-phenol red being used as indicator.

The results were as follows: *With* Co^{++}, ammonia in 10 ml. was found equivalent to 6.70 ml. of HCl (0.02 N) after correction by subtraction of a blank (0.23 ml.) obtained in a control in which arginine alone was omitted; without Co^{++}, 3.65 ml. (corrected). In a control in which arginase was omitted (Co^{++} and arginine present) and urea, 10.0 mg., added immediately before the 2 hour incubation at 37°, the acid *found* equivalent to NH_3 (from 10 ml. of filtrate) was 6.61 ml. (corrected), the *calculated* amount (*i.e.* equivalent to 4 mg. of urea) being 6.67 ml.; the correction was applied by subtraction of 0.47 ml., obtained in a similar control with urea absent.

Discussion of Method—The development of details of the method need not be discussed at length. Some of the factors considered will be treated briefly. Arginase solution from recently prepared arginase powder was usually found to be more potent (when non-activated by Co^{++}, etc.) than the extracts of older preparations; however, the maximal activities (activated enzyme) remained fairly constant for months. Some variations are, therefore, to be anticipated in the activity of different crude preparations and in preparations of varying ages.

In the typical determination (cited above), phosphate in the reaction mixture was about M/3. Increase of the phosphate concentration to $\frac{2}{3}$ M, other conditions being unchanged, resulted in a decline of about 15 per cent in apparent activity; enhancement of activity accompanied a decrease to 0.1 M in the phosphate concentration. A systematic determination of the effect of ionic strength was not of *immediate* importance. In a series of experiments in which veronal (23) buffer (pH 7.5), present in a concentration of 0.07 M, was substituted for phosphate buffer of the same pH and concentration, the enzyme activity was not significantly changed. However, specific and general salt effects may become of great moment, as is demonstrated by the observations of Hunter *et al.* (19) of the marked depression of the activity by borate in comparison with the effects of certain other buffers (pH, unchanged); similar results were obtained with this arginase. The relation of pH to arginase activity has already been evaluated (18, 19). For work at pH 9.5, we used Sörensen's glycine buffer, taking due account of the potential reactivity of glycine toward various reagents (*e.g.* oxidizing agents). The arginine salts used were preparations from gelatin, made and purified in this laboratory, or high grade commercial products.

Sodium bisulfite (used especially when quinone had been previously added, and not necessarily otherwise) and sodium cyanide were added in order to protect urease against the action of various reagents; *e.g.*, excess quinone, metal ions, etc. (*cf.* (1, 2)). It was amply demonstrated by means of suitable controls that the accuracy of the urea estimations was not impaired. An additional, indispensable function of the cyanide will be discussed later.

Fosse's xanthydrol method (24) for the estimation of urea as adapted to arginase determinations by Karrer and Zehender (25), was found of some utility for qualitative purposes but of limited value for the quantitative needs of this work. Although no precipitation of dixanthydryl urea was obtained from mixtures in which hydrolysis of arginine had not taken place, the weight of this derivative obtained from hydrolyzed material was occasionally far in excess of that anticipated on the basis of parallel urease determinations. Such dixanthydryl urea was found by analysis (N) to be impure and to contain ash.

DISCUSSION OF RESULTS

A detailed account of the numerous individual experiments underlying this work will not be attempted. A few representative data are tabulated to illustrate the magnitude of the effects observed under specified conditions. The experimentation has necessarily been guarded at every stage by adequate control determinations.

It should be emphasized at once that comparisons of the results of different investigators in this field must take account of the varying experimental conditions employed. Arginase preparations are at best crude mixtures, varying greatly in their content of extraneous material. This material contributes to the activation picture and modifies the effects of added reagents. The hydrogen ion concentration of the reaction mixture necessarily influences the action of many reagents. Specific effects of a buffer salt may not be predictable. For example, ferr*ic* ion or selenious oxide abolishes the activity of our preparation when added to the enzyme before, but not after, the addition of phosphate buffer (pH 7.5); cobaltous ion activates regardless of the order of addition. Much of the arginase work already reported rests upon determinations of activity in glycine buffer at pH 9 to 9.5, presumably because this is near the pH range of optimal activity for arginase. The preparation used by us was found sufficiently active in phosphate of pH 7.5, a less "favorable" region, where, however, the enzyme is more stable (19) and the effects of many added reagents are apparently accentuated. Most of our results are based upon work in the lower pH range, although comparisons were frequently made at pH 9.5.

Activating Effects of Metal Ions—The activating effect of ferrous ion upon arginase has already been emphasized by several authors. It has been shown that this activation is often more reliable and pronounced when the Fe^{++} is used together with organic thiol

TABLE I

Effects of Reagents upon Arginase Activity; Activations, Inactivations, and Reactivations by Specified Reagents

The procedure was essentially the same as for the *typical experiment* described under "Methods;" exceptions are noted. Activities are expressed as ml. of 0.02 N HCl (corrected), equivalent to NH_3 present in 10 ml. of filtrate after urease action (10 ml. = two-fifths of the reaction mixture). Corrections were applied on the basis of controls (*cf.* "Methods").

Arginase solution*	0.02 N HCl corresponding to initial activity	Reagents added	0.02 N HCl corresponding to activity
	ml.		*ml.*
A to F†	3.42	$CoCl_2 \cdot 6H_2O$	6.35
G	3.06	$MnSO_4 \cdot 4H_2O$, 2 mg.	5.56
Same		$Ni(NO_3)_2 \cdot 6H_2O$, 3 mg.	5.61
H‡	4.19	$Co(NO_3)_2 \cdot 6H_2O$, 3 "	6.00
Same		$ZnSO_4 \cdot 7H_2O$, 3 mg.	3.56
I§	5.02	Cysteine HCl (neutralized), 6 mg.	4.31
Same		I_2, 0.01 N, 0.6 ml.	0.00
"		Same + $CoCl_2 \cdot 6H_2O$, 3 mg.	7.29
J‖	3.65	$CoCl_2 \cdot 6H_2O$, 3 mg.	6.46
Same		$NaHSO_3$, 5 mg.	3.34
"		Benzoquinone, 1 mg.	0.34
"		Same + $NaHSO_3$, 5 mg.	0.94
"		" + $CoCl_2 \cdot 6H_2O$, 3 mg.	4.87
"		" + $Ni(NO_3)_2 \cdot 6H_2O$, 3 mg.	3.73
"		" + $FeSO_4 \cdot 7H_2O$, 3 mg.	0.28
"		" + cysteine HCl (neutralized), 6 mg.	1.71
"		" + " " + $FeSO_4 \cdot 7H_2O$, 3 mg.	3.94
K	3.60	$Fe_2(SO_4)_3$, 2 mg.	0.20
"		Same + $CoCl_2 \cdot 6H_2O$, 3 mg.¶	2.51
"		$Fe_2(SO_4)_3$, 2 mg.**	2.47
L	5.90	C_6H_5HgOH, 3 mg. + $C_6H_5CH_2HgCl$, 3 mg.††	5.79
M‡‡	3.86	$K_3Fe(CN)_6$, 10 mg.	2.24
"		$K_3Fe(CN)_6$, 3.3 mg.	2.48
"		$K_4Fe(CN)_6 \cdot 3H_2O$, 4.2 mg.	3.65
"		$Ni(NO_3)_2 \cdot 6H_2O$, 2 mg. + $K_3Fe(CN)_6$, 3.3 mg.	5.23
"		$MnSO_4 \cdot 4H_2O$, 2 mg. + $K_3Fe(CN)_6$, 3.3 mg.	4.37
N§§	3.00	H_2S water (saturated; about 0.1 M), 5 ml.	0.75
Same		Same + N_2 treatment	1.85
"		" + " " + $CoCl_2 \cdot 6H_2O$, 3 mg.	6.89

* Arginase solutions are arbitrarily lettered, for convenience.

TABLE I—*Concluded*

† Average of six determinations; the enzyme solution in each instance was freshly prepared from the same stock sample of powdered arginase; initial activities varied, 3.12 to 3.67 ml.; with cobalt (0.1 to 3 mg.), 6.44 to 6.85 ml. Magnitude of corrections: determination with cobalt, enzyme omitted, 0.33 ml.; with cobalt, *boiled enzyme solution* present, 0.43 ml.

‡ Deviations from *typical determination*: arginase solution 1.0 ml., glycine buffer (0.02 M, pH 9.5) 10 ml., instead of phosphate in the initial mixture; cobalt or zinc salt added 15 minutes before the addition of buffer.

§ The initial reaction mixture to which reagents were added consisted of 5 ml. of arginase, 4 ml. of water, and 10 ml. of phosphate (1 M, pH 7.5); the volume of the arginase-arginine digestion mixture, 21 ml.; the reagent first added was permitted to act 30 minutes before further additions. The addition of cysteine with Co^{++} (after I_2), in a parallel determination, introduced no observable change from the effect of Co^{++} alone; "activity" when Co^{++} was added directly to the initial mixture, 8.24 ml. (93 per cent hydrolysis); for Ni^{++} instead of Co^{++}, 7.77 ml. When urea, 10.0 mg., was added after iodine and $NaHSO_3$, 10 mg. (arginine present and arginase omitted), the results were: found, 6.53 ml.; calculated for 4.0 mg. of urea (≏ 10 ml. of urease-filtrate), 6.67 ml. The magnitude of reactivation from iodine by nickel or manganous salts, or $FeSO_4 \cdot 7H_2O$ (added as a solid after the addition of some cysteine), was of the order of 60 to 75 per cent of the cobalt effect. Cysteine, alone, occasionally effected slight *re*activation.

‖ Each added reagent was permitted to act 20 minutes before any further addition. Control determinations of urea in the presence of arginine and various added reagents (arginase omitted) gave satisfactory results.

¶ Ferric salt added before phosphate; phosphate added before cobalt addition.

** Added *after* phosphate.

†† Added to arginase, 5 ml.; the mixture stood 1 hour before the addition of 10 ml. of phosphate.

‡‡ Reagents were added (after phosphate) in the order named in intervals of 20 minutes; somewhat similar effects were observed when glycine (pH 9.5) buffer was used after addition of reagents to the enzyme, but the magnitude of the changes was less.

§§ The second and third digestions were in pure N_2 (*cf.* "Other reagents").

compounds or certain other substances with which it might combine to form ferrous complex ions. Such results have also been obtained in this work. This action of ferrous ion is usually interpreted in terms of a reduction. It seemed to us that this interpretation might require modification in view of our observations of the failure of reducing agents of greater potency, such as HCN,

H_2S, organic thiol compounds, etc., to activate under varying conditions. Thus, the effect might be related to some other attribute of the ferrous ion, specifically the property of coordination (*cf.* (26)) with suitable molecules (*e.g.* derivatives of NH_3). This conception, which involves no unique property of ferrous ion, prompted experimentation with a variety of ions, with the result that ions of the other metals of the iron triad (Co^{++} and Ni^{++}) and, in addition, Mn^{++} (*cf.* (27)) were found to be excellent activators. Typical results are recorded in Table I. The arginase in freshly prepared enzyme powder is more active than in older preparations, but the enzyme in preparations several months old is extensively activated in the presence of these ions. The effects are less pronounced at pH 9.5 (glycine buffer) than at pH 7.5, and this is especially true for Ni^{++}. Cobaltous ion appears to be the most potent activator. The ions, Cd^{++} and Zn^{++}, exhibit no comparable effect; Cd^{++} possibly activates slightly, while Zn^{++} is inert or actually depressant (*e.g.* at pH 9.5). Certain ions abolish the activity, apparently in part by precipitation of enzyme substance. Examples are Cu^{++}, Hg^{++}, Ag^{+}, and $PtCl_6^{=}$. Partial restoration from the action of Cu^{++} is occasionally effected by removal of the cupric copper by means of the prolonged action of a zinc-platinum couple. In such instances, the further addition of Co^{++} results in increased reactivation.

From controlled inactivations of arginase by quinone, iodine, or ferric ion, the activity is readily restored and usually maintained at its maximum by suitable addition of cobaltous ion. Ni^{++} and Mn^{++} act similarly but in different degree.

Magnitude of Activation by Co^{++}—This is most conveniently gaged by a comparison of the relative rates of hydrolysis of arginine, catalyzed by arginase with and without Co^{++}. Table II records some preliminary results. Such observations are being extended; their implications for arginase action need not be discussed here. It may be noted that, in the determinations *with cobalt*, the reaction constant, calculated on the basis of an uncomplicated pseudounimolecular reaction, exhibits over the range $t = 31.5$ to 181.5 minutes (arginine, about 85 per cent hydrolyzed) a rather small deviation from the average value. This may be somewhat fortuitous with the few data presented, but is certainly less true for the hydrolysis *without cobalt*. The data afford a basis for rough comparison, and show, further, that under the conditions the hydrolysis, *with cobalt*, is apparently nearer completion after 20 hours or less than after 48 hours, *without cobalt*.

These observations suggest possible advantages in the use of cobaltous chloride with arginase, under proper conditions, in certain estimations of arginine, *e.g.* in biological material.

TABLE II

Magnitude of Activation by Cobaltous Ion

The procedure was similar to that of the *typical experiment* described under "Methods." The determination after 121.5 minutes was inadvertently omitted in the series *with cobalt*. Each result represents an independent determination; all determinations were started on the same day, one preparation of arginase solution serving as source of enzyme for all.

The reaction constants, K and K_{Co}, were calculated rather empirically with the use of the equation $K = 1/t \ln(a/a - x)$; time, t, is expressed in minutes; for a is used 8.66, the number of ml. (corrected) of 0.02 N HCl *found* equivalent to ammonia in 10 ml. of urease-filtrate after 48 hours hydrolysis of arginine (cobalt present), as compared with 8.82, ml. *calculated* (complete hydrolysis of the arginine used); for x is used the corresponding number of ml. (corrected) of 0.02 N HCl found equivalent to urea set free during time of arginine hydrolysis, t.

Time of hydrolysis	Arginase (non-activated)		Arginase + $CoCl_2 \cdot 6H_2O$	
	0.02 N HCl equivalent to urea formed	K (calculated)	0.02 N HCl equivalent to urea formed	K_{Co} (calculated)
min.	*ml.*		*ml.*	
31.5	1.39	0.0056	2.43	0.0104
61.5	1.81	0.0038	4.01	0.0101
121.5	3.31	0.0040		
181.5	4.12	0.0036	7.45	0.0108
hrs.				
20	7.10		8.67	
48	8.24		8.66	

Other Characteristics of Activation by Co^{++}—Series of parallel determinations of arginase activity, influenced by $CoCl_2 \cdot 6H_2O$ added to the reaction mixtures *seriatim* in specified increments from 0.001 mg. to 6.0 mg. show that the minimum concentration of Co^{++} for maximal activation *under the conditions* is of the order 1.5×10^{-5} M. This is illustrated by typical data of Table III. The corresponding optimal concentration for Ni^{++} may be placed at about 2×10^{-4} M (a preliminary value). The ions (without arginase) do not as such display catalytic properties under the conditions; this is proved by controls and by series of experiments in which Co^{++} concentration is held above the optimal and the amounts of arginase varied, from which it appears that the catalytic action is directly proportional to the amount of arginase added, with or without Co^{++}; the data are actually more consistent when Co^{++} is present.

TABLE III

Activation of Arginase in Relation to Cobaltous Ion Concentration

Procedure—The specified amount of cobalt chloride in 1.0 ml. of water, added to arginase solution 0.5 ml., water 10 ml., and phosphate (1 M, pH 7.5) 5 ml.; the mixture stood 20 minutes before the addition of arginine carbonate 45 mg., in water, 1.0 ml.; digestion time, 3 hours; $NaHSO_3$ (but not NaCN)* omitted. The data are recorded in the two left-hand columns; initial activity, expressed as ml. of 0.02 N HCl, 1.69. In the two right-hand columns are recorded data from a similar series, by the same procedure except that arginase solution, 2.0 ml., was used and the digestion time was 2 hours; initial activity, 3.97.

$CoCl_2 \cdot 6H_2O$ added	0.02 N HCl equivalent to urea formed	$CoCl_2 \cdot 6H_2O$ added	0.02 N HCl equivalent to urea formed
mg.	*ml.*	*mg.*	*ml.*
1.0	4.01	6.0	6.34
0.1	3.79	2.0	6.44
0.05	3.72	1.0	6.44
0.01	2.57	0.1	6.41
0.005	2.19	0.05	6.39
0.001	1.59	0.01	5.39

* Parallel determinations: (1) arginase solution *boiled* before use (or omitted altogether), and NaCN omitted; found, 5.53 ml.; (2) the same, but arginine also omitted; found 0.21 ml. This illustrates the arginine-metal-urease phenomenon (*cf.* Table IV and "Discussion") and indispensability of NaCN.

Inactivations by Oxidizing Agents; Reactivations by Certain Metal Ions—Such oxidants as methylene blue and dipotassium indigodisulfonate exhibited no observable effect upon the activity of arginase. Iodine or quinone drastically suppressed the activity. From *controlled* inactivations by these reagents the activity was, apparently, extensively recovered in the presence of Co^{++} especially and, perhaps, to a lesser degree, of Ni^{++} and Mn^{++} as well as Fe^{++}. The reactivating effect of Fe^{++} was dependable only when its use was preceded by that of some suitable reagent (*e.g.* cysteine). The other ions were effective when added alone. Reactivation from quinone was realized *to a limited degree* by the use of cysteine or bisulfites. Arginase activity was abolished by selenium dioxide (apparently irreversibly) and by ferr*ic* ion only when the addition to the enzyme of these reagents preceded the addition of phosphate; from inactivations by Fe^{+++}, the activity was readily restored by Co^{++}. The action of the system, $Fe(CN)_6^{\equiv}:Fe(CN)_6^{\equiv}$, involved a noteworthy peculiarity. Ferrocyanide did not alter the enzyme activity significantly; depression resulted increasingly as the ratio of ferricyanide to ferrocyanide was increased in mixtures of these ions added to the enzyme; but complete inactivation of arginase was not readily effected, even by the use of the oxidant $Fe(CN)_6^{\equiv}$ alone. When a sufficient amount of Ni^{++} or Mn^{++} was also present, the activity was not depressed. Table I shows typical results.

Other Reagents—Bisulfites and organic thiol compounds did not activate this arginase; cysteine depressed the activity somewhat. The supporting action of cysteine, when used with Fe^{++}, has been cited. It has been plausibly suggested (28) that such behavior is related to the maintenance in reaction mixtures of Fe^{++} in the reduced state. So potent a reductant as $TiCl_3$ (in borate buffers) was found incapable of enhancing arginase activity under conditions where Co^{++} effected an increase. When these and other reagents were added to arginase, appropriate measures were taken to avoid injury to the enzyme by an inadvertent alkalinization or acidification.

The action of HCN upon arginase at pH 7.5 was, in general, depressant (*cf.* (10)). At no time was activation by HCN observed.

Hydrogen sulfide markedly depressed arginase activity (at pH 7.5) *but partial restoration from the action of this reagent could be effected by simple removal of the excess* in a stream of air or nitrogen or by precipitation of sulfide ions with Zn^{++}. The further addition of Co^{++} ions established more extensive reactivation. Such results were also obtained when the reactions were carried out in an atmosphere of pure nitrogen. For this purpose, buffered arginase solution, alone or treated with H_2S, and arginine solution, with or without the addition of Co^{++}, were separately swept with nitrogen (passed over CuO and Cu in the usual manner) in special all-glass apparatus, so constructed that the solutions could be mixed in the nitrogen atmosphere and the mixture maintained therein during the digestion and the subsequent destruction of the enzyme by heat. Arginase action can evidently take place in the absence of air and the *activations* observed are also, *apparently*, independent of an oxygen effect (*cf.* (15, 27, 28)).

The presence in reaction mixtures of mercaptide-forming organomercurials, such as phenylmercuric hydroxide and benzylmercuric chloride, had no observable effect upon the action of arginase (*cf.* (2)).

Urease, Metals, and Arginase Activity

From the inception of this work, we adopted the practice of adding sodium cyanide to the buffered reaction mixtures immediately before the addition of urease in the estimations of urea formed in the preceding step (hydrolysis of arginine). The primary purpose was to insure protection of the urease against a possible destructive action of some of the reagents (salts or organic derivatives of heavy metals) added earlier. For example, certain heavy metal ions would be bound as complex cyanide ions. Secondarily, cyanide might serve to inactivate any residual traces of arginase. Further, the presence of cyanide is favorable to urease action (1).

In the later development of the work we were led by certain theoretical considerations to test, as possible catalysts for the hydrolysis of arginine (independent of arginase), mixtures of one of the ions, Co^{++}, Ni^{++}, Mn^{++}, with various synthetic nitrogen compounds that are known to coordinate with such ions. Under these conditions, the extensive hydrolysis of arginine was indeed observed, but a careful analysis of the situation soon disclosed that urease was in some manner involved. This was made evident by the following: (1) increase in the amount of cyanide added before the addition of urease or decrease in heavy metal ion concentration (and omission of any synthetic material) abolished the effect; (2) when arginase and NaCN were both omitted in the typical determination with Co^{++} (described in a preceding section), there was observed extensive hydrolysis of arginine. It is concluded that significant hydrolysis of arginine *in the absence of arginase* results when urease and cobaltous ion are both present in the reaction mixture.

Subsequent investigation proved that Co^{++} may be replaced by Ni^{++}, Mn^{++}, or (apparently) Fe^{++} plus cysteine. Indeed, with some samples of crystalline urease, particularly at a higher pH level, appreciable hydrolysis is observed without added metal. Does such urease "contain" effective metal (*e.g.*, as impurity) or does it function as such simply by the usual catalytic action upon urea hydrolysis, effecting the removal of one of the products of arginine hydrolysis? Further experimentation should clarify these and many other matters requiring explanation.

The urease-metal effect described was observed alike with crude jack bean urease and crystalline urease (29). Efforts to refer the effect to some constituent of jack bean other than urease have been unsuccessful.

Table IV records some of the results.

Sodium cyanide, as applied in this investigation, apparently serves effectively to inhibit this urease-metal action. Its indispensability in the evaluation of arginase activity in this investigation needs no further comment. The bearing of these results upon analytical operations involving in general the use of urease in the determination of urea, where arginine and certain metal ions may also be present, is obvious.

Theoretical

The experimental results emphasize certain noteworthy characteristics of arginase activation. Some of these are (1) the failure of several potent reducing agents, *e.g.* those that activate papain, to activate partially inactivated arginase, or to effect, under the conditions used, the extensive restoration of the activity after controlled inactivations by oxidants; (2) the competency of certain metal ions, *e.g.* Co^{++}, Ni^{++}, Mn^{++}, to effect such activations or reactivations; (3) the depressant or inactivating effect of HCN and H_2S, in contrast to the favorable action of these reagents upon papain or urease activity.

There are significant objections to an interpretation of the observed action of an ion like Co^{++} in terms of a reduction. In Table V are listed for comparison recorded values of the potentials of $Co^{+++}:Co^{++}$ and other "positive" systems with those of several less "positive" electromotively active systems. In so far as it is *permissible* to apply such data here it may be said that it is extremely unlikely that the reductant ion of so positive a system as $Co^{+++}:Co^{++}$ might, of *itself*, function to reduce an enzyme. The

TABLE IV

Hydrolysis of Arginine in Presence of Urease and Certain Heavy Metal Ions

Crystalline urease prepared from 100 gm. of jack bean meal was dissolved in 25 ml. of water. Determinations were conducted as follows: metal salt was added to the mixture, urease solution 5 ml., water 10 ml., and phosphate buffer (1 M, pH 7.5) 3 ml.; after 20 minutes some caprylic alcohol and arginine carbonate, 45 mg., were added; digestion, 20 hours at 30°; 3 ml. of phosphate were added and the mixture held 10 minutes at 100°, and then brought to 25 ml.; NH_3 was determined in 10 ml. Results are expressed as ml. of 0.02 N HCl equivalent to NH_3.

Reagent added	0.02 N HCl
	ml.
None	3.76*
$CoCl_2 \cdot 6H_2O$, 2 mg	8.72†‡
$Ni(NO_3)_2 \cdot 6H_2O$, 3 mg	8.76
$MnSO_4 \cdot 4H_2O$, 3 mg	8.67
Cysteine hydrochloride (neutralized) + $FeSO_4 \cdot 7H_2O$, 3 mg	6.83
C_6H_5HgOH, 5 mg. + $CoCl_2 \cdot 6H_2O$, 2 mg	0.19§

* Parallel determinations: (1) crude urease (22), 0.1 gm., substituted for crystalline urease, HCl, 0.32 ml.; (2) digestion time (crystalline urease used), 2 hours (instead of 20 hours), HCl 0.17 ml.

† Controls: (1) boiled urease solution used and all other factors unchanged, HCl 0.10 ml.; (2) arginine (only) omitted, HCl 0.05 ml.

‡ Parallel determinations: (1) crude urease, 0.1 gm., substituted for crystalline urease, HCl 8.82 ml.; (2) digestion time (crystalline urease used), 2 hours (instead of 20 hours), HCl 1.96 ml. Controls with crude urease: no NH_3 obtained when only urease or arginine was omitted; nor when cobalt was present and urease or arginine was omitted.

§ C_6H_5HgOH *inactivates urease* (*cf.* (1)) but does not inhibit "true" *arginase* activity (*cf.* Table I); application of the xanthydrol test failed to detect urea in the final mixture.

limited degree of inactivation exhibited by ferricyanide ion is of interest in this connection. It has been shown that cobaltous ion reactivates arginase, initially treated with inhibiting ferric ion. This can scarcely be "reduction" in the accepted sense. Finally, the failure of certain common reducing agents to reactivate has already been pointed out.

The depressant action of HCN and H_2S upon arginase suggests that the enzyme, itself, may partake of the nature of a heavy metal complex (*cf.* (10)). If such a suggestion is tenable, there is provided a basis for the correlation of most of the facts observed. If the metallic component of such a complex molecule is present in a reduced state when the enzyme is active, the following might be assumed. Inactivation by oxidants might involve (1) simple oxidation of the complex, reversed by suitable reduction; or (2) separation of the metallic ion or component from the remainder of the molecule, possibly coincident with the oxidation of the former. The second, more drastic event, would correspond to usual experience. If so, suitable reduction might, under favorable circumstances, be accompanied by recombination of the enzyme fragments. However, *partial* reactivations are only occasionally brought about by reductants, while reactivation by Co^{++}, etc., is more dependable and complete.

TABLE V

Potentials of Various Oxidation-Reduction Systems*

System	Volts	System	Volt
$Co^{+++}:Co^{++}$	1.81	$Fe^{+++}:Fe^{++}$	0.747
$Ce^{++++}:Ce^{+++}$	1.5	$\frac{1}{2} I_2:I^-$	0.534
$\frac{1}{2} Cl_2:Cl^-$	1.36	$Fe(CN)_6^{\equiv}:Fe(CN)_6^{\equiv}$	0.486

* Washburn, E. W., International critical tables of numerical data, physics, chemistry and technology, New York, 6, 332 (1929).

The depression of activity by hydrogen sulfide might conceivably result (*a*) by the formation of a loose addition compound with the metal-enzyme, from which the enzyme is regenerated by suitable removal of H_2S; or (*b*) by disruption of the metal-enzyme and, possibly, conversion of the metallic component to a sulfide. It has been shown that a partial regeneration of activity is, indeed, readily obtained. It seems significant that, in this instance also, extensive restoration has been effected by the suitable addition of cobaltous ion.

A rather plausible explanation of the activating property of the effective ions, Co^{++}, Ni^{++}, Mn^{++}, or Fe^{++}, rests upon the assumption that such ions (probably maintained in their reduced states) may replace, more or less readily, the metallic atom or component, characteristic of arginase, when that component has been lost to the enzyme by some process which has not injured the rest of the molecule. Such reactivation by "substitution" would be intimately related to the very characteristic property of these ions to form metal-coordinated complexes (*e.g.* the so called metal ammines). The metal ammines derived from reduced ions (Me^{++}) are often less stable than their oxidized analogues. Their existence is, however, unquestioned. The assumed coordination in the activation picture might be conceived as involving both enzyme and substrate through contributions of suitable groupings by each (*e.g.* a portion of the guanido group of arginine). This assigns to the metal an interesting rôle in the building of an enzyme-substrate compound. It is conceivable that such coordination would eliminate resonance in the positively charged component (*i.e.* the guanidinium grouping) of the arginine zwitter ion. Molecular resonance has been plausibly assumed to exist (30) in the ion formed by addition of H^+ to guanidine or a guanidino group. In the present instance, the "fixing" of bonds in the guanido group through enzyme-metal coordination might be reflected in promotion (acceleration) of hydrolysis in the direction of formation of ornithine and urea, with concomitant regeneration of the metal-enzyme. This hypothetical mechanism of arginase action may possibly find application in the further study of the arginine-metal-urease phenomenon already described.

SUMMARY

With the enzyme preparation used, it has been found that arginase, under the experimental conditions, is neither activated nor, after treatment with various oxidizing agents, reactivated by cysteine and other reducing agents that are known to activate papain. Arginase may, however, be effectively activated, or reactivated after treatment with oxidants, by certain metal ions, specifically Co^{++}, Ni^{++}, or Mn^{++} (as well as Fe^{++}). Cobaltous ion effects also the complete restoration of arginase activity after the enzyme has been inactivated by hydrogen sulfide and the excess of the latter removed.

Organomercurials of the type RHgX do not inactivate; ferricyanide ion suppresses the activity incompletely; and the ions, Cu^{++}, Hg^{++}, and Ag^{+} destroy the activity, in part, by precipitation of the enzyme substance. The action of HCN is depressant.

The magnitude of activation by cobaltous ion is illustrated by appropriate rate measurements; certain other characteristics of the activations are also indicated.

On the basis of the results, the arginase molecule is visualized as containing a metallic component which may be oxidized, or separated from the rest of the molecule by the action of oxidizing agents or certain other reagents. The observed actions of cobaltous ion and the other effective ions are interpreted, not as reductions, but rather in terms of the characteristic property of these ions to coordinate with suitable molecules or groupings to form complex molecules. This suggestion may include an implication regarding the rôle of the metallic component in the building of an enzyme-substrate compound.

Arginine, without arginase, is hydrolyzed in the presence of one of the ions, Co^{++}, Mn^{++}, Ni^{++}, and under certain conditions, Fe^{++}, when urease is also present in the reaction mixture. Organometallic suppressors of urease activity also suppress this effect. Cyanide diminishes the arginine-metal-urease effect, but, being a urease activator, does not prevent the independent hydrolysis of any urea also present in the reaction mixture. The bearing of these results upon the conduct of certain analytical operations is indicated.

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Amer. Jour. Clin. Nutrition 8(4): 855-863 (1960)

Trace Metals in Human Plasma and Red Blood Cells

A Study of Magnesium, Chromium, Nickel, Copper and Zinc
II. Observations of Patients with Some Hematologic Diseases

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In part I of this study, plasma and red blood cell values for magnesium, chromium, nickel, copper and zinc in normal persons are reported. The observations made of a group of patients with hematologic diseases (see Tables I and II) are reported herein.

Patients were chosen for study from the wards and Hematology Clinic of the University of Virginia Hospital. Only patients with unequivocal diagnoses were selected, and whenever possible blood samples were taken before and after treatment. The presence of complications and concurrent diseases resulted in exclusion of several patients because of the possible introduction of additional variables. The spectrochemical method of analysis was employed throughout.

MAGNESIUM

It now seems established that alterations in the content of magnesium in the body are capable of producing clinical manifestations. Since magnesium, like potassium, is chiefly an intracellular ion, the serum level is a poor measure of total deficit or excess. Doubtlessly this factor has delayed recognition of the association of magnesium with certain clinical states. Smith and Hammarsten have demonstrated this by a recent study. Of twelve patients with delirium tremens, the levels of magnesium in plasma were below the normal range in only seven, whereas values for the red blood cells were decreased in all of the patients.[1,2] Previous studies of patients with this condition have dealt only with serum values,[3,4] and have been judged inconclusive.[5] In delirium tremens the administration of magnesium is generally followed, after some delay, by improvement.[1–3]

Spontaneous normocalcemic, hypomagnesemic tetany has been reported.[6] Other conditions in which magnesium depletion may be implicated are heart failure treated with ammonium chloride and mercurial diuretics, chronic nephritis,[7] treated diabetic acidosis,[8] replacement of large fluid losses (e.g., by intubation) with fluids poor in magnesium,[2,9] and severe burns.[10] Osteolytic bone lesions with hypercalcemia and hypercalciuria,[2] hyperparathyroidism,[11,12] and primary hyperaldosteronism[13,14] have been associated with very low levels of magnesium in the serum. Reports of changes in uncomplicated portal

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Supported in part by grants No. H-1102 and H-1621 from the National Institutes of Health.

Table I

Plasma Values for Patients with Some Hematologic Diseases*

Disease	No. of Samples and Condition	Magnesium	Chromium	Nickel	Copper	Zinc
None	109 (normal)	20.4 (12.5–36.0)	0.027 (0.009–0.055)	0.060 (0–0.27)	1.03 (0.48–1.93)	3.01 (0.49–7.70)
Aplastic anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	17.5 (16.3–18.1)	0.022 (0.022–0.023)	0.013 (0–0.040)	1.17 (0.86–1.71)	2.27 (1.30–3.00)
Myelofibrosis	0 (untreated)	...	...	...	...	...
	3 (treated)	21.8 (17.3–23.2)	0.024 (0.020–0.033)	0.027 (0–0.040)	0.99 (0.87–1.09)	2.50 (1.40–3.10)
Acquired hemolytic anemia	1 (untreated)	20.7	0.027	0.040	0.68	2.50
	4 (treated)	17.0 (14.3–21.7)	0.021 (0.020–0.024)	0.017 (0–0.070)	1.16 (0.64–2.30)	2.65 (1.60–4.20)
Sickle cell anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	19.2 (13.3–28.5)	0.023 (0.021–0.028)	0.043 (0–0.130)	1.67 (0.95–2.20)	2.71 (1.70–4.20)
Pernicious anemia	7 (untreated)	18.6 (12.0–26.9)	0.025 (0.012–0.041)	0.053 (0–0.10)	1.21 (0.90–1.60)	1.19 (0.64–2.00)
	15 (treated)	17.6 (14.2–22.2)	0.030 (0.019–0.058)	0.051 (0–0.130)	1.02 (0.50–1.55)	3.88 (2.40–7.80)
Iron deficiency anemia	6 (untreated)	20.4 (16.4–22.8)	0.029 (0.020–0.028)	0.017 (0–0.100)	1.42 (0.82–2.04)	3.60 (2.25–4.50)
	1 (treated)	20.9	0.032	0	0.96	6.10
Polycythemia vera	3 (untreated)	21.2 (14.4–30.0)	0.043 (0.027–0.072)	0.110 (0–0.230)	1.67 (0.77–3.20)	3.90 (3.00–5.10)
	9 (treated)	18.6 (12.7–22.6)	0.029 (0.015–0.037)	0.044 (0–0.120)	1.24 (0.54–2.10)	3.80 (1.80–6.10)
Acute leukemia	3 (untreated)	21.2 (16.4–28.7)	0.026 (0.024–0.029	0.013 (0–0.040)	1.56 (0.67–2.06)	4.03 (3.55–4.65)
	6 (treated)	22.2 (16.4–25.8)	0.023 (0.011–0.030)	0.013 (0–0.040)	1.60 (0.94–2.30)	2.89 (1.57–4.90)
Chronic myelogenous leukemia	1 (untreated)	18.6	0.016	0.10	0.88	1.40
	4 (treated)	17.7 (13.1–24.1)	0.025 (0.020–0.035)	0 (0)	0.71 (0.51–0.87)	2.44 (1.50–3.80)
Chronic lymphocytic leukemia	2 (untreated)	19.7 (19.3–20.0)	0.029 (0.023–0.035)	0 (0)	1.15 (1.06–1.23)	3.90 (3.20–4.60)
	4 (treated)	23.4 (15.8–33.3)	0.027 (0.020–0.034)	0.042 (0–0.130)	1.49 (1.19–1.95)	2.98 (2.20–3.50)
Hodgkins disease	1 (untreated	11.5	0.018	0	1.34	1.60
	11 (treated)	18.2 (12.4–28.5)	0.027 (0.018–0.039)	0.027 (0–0.150)	1.48 (0.72–4.50)	3.35 (1.50–7.10)
Lymphosarcoma	2 (untreated)	20.3 (16.8–23.8)	0.022 (0.021–0.023)	0.020 (0–0.040)	1.32 (1.14–1.51)	2.38 (1.75–3.00)
	14 (treated)	19.0 (13.5–26.0)	0.028 (0.019–0.043)	0.024 (0–0.180)	1.45 (0.60–2.90)	3.20 (1.50–6.00)
Multiple myeloma	5 (untreated)	21.8 (15.9–29.0)	0.023 (0.017–0.027)	0.054 (0–0.130)	1.19 (0.81–1.87)	2.62 (0.72–4.40)
	3 (treated)	19.4 (15.1–27.0)	0.024 (0.022–0.026)	0.013 (0–0.040)	0.91 (0.77–1.00)	3.73 (3.10–4.20)

* Mean values and ranges are given in p.p.m. (μg. per gm.). Ranges are in parentheses.

cirrhosis and diabetes mellitus are conflicting.[2,4,15]

Hypermagnesemia has long been associated with renal failure, although it may not be observed even with severe nitrogen retention. Smith and Hammarsten found the magnesium levels in the plasma elevated in only ten of fourteen patients with uremia and depression of the central nervous system, but the red blood cell value was high in all subjects, again emphasizing the importance of the intracellular level.[1,2] Hamburger found the levels of

TABLE II
Red Blood Cell Values for Patients with Some Hematologic Diseases*

Disease	No. of Samples and Condition	Magnesium	Chromium	Nickel	Copper	Zinc
None	106 (normal)	74.3	0.021	0.053	0.82	10.0
		(26.0–131.0)	(0.005–0.054)	(0–0.31)	(0.28–2.80)	(3.80–25.40)
Aplastic anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	72.0	0.021	0.053	0.68	9.20
		(57.7–80.6)	(0.015–0.026)	(0–0.080)	(0.49–0.84)	(6.20–13.20)
Myelofibrosis	0 (untreated)	...	...	...	...	...
	3 (treated)	143.0	0.021	0.080	0.68	9.33
		(117.0–181.0)	(0.018–0.025)	(0.055–0.115)	(0.55–0.87)	(8.00–11.70)
Acquired hemolytic anemia	0 (untreated)	...	...	...	...	...
	4 (treated)	95.3	0.024	0.017	0.51	9.43
		(73.3–136.7)	(0.016–0.035)	(0–0.030)	(0.50–0.72)	(5.90–12.80)
Sickle cell anemia	0 (untreated)	...	...	...	...	...
	2 (treated)	62.2	0.036	0.080	0.90	17.70
		(53.2–71.3)	(0.032–0.039)	(0.020–0.140)	(0.74–1.06)	(15.90–19.50)
Pernicious anemia	6 (untreated)	90.9	0.022	0.070	0.81	8.23
		(52.5–151.0)	(0.017–0.033)	(0–0.210)	(0.54–1.06)	(4.80–10.50)
	14 (treated)	86.3	0.026	0.032	0.87	11.08
		(48.8–146.1)	(0.015–0.041)	(0–0.100)	(0.32–1.43)	(4.70–20.60)
Iron deficiency anemia	6 (untreated)	80.7	0.026	0.015	0.73	11.70
		(50.7–133.2)	(0.012–0.052)	(0–0.050)	(0.64–0.81)	(8.00–15.10)
	1 (treated)	57.6	0.025	0.020	0.52	14.80
Polycythemia vera	3 (untreated)	59.0	0.039	0.085	0.73	10.00
		(32.5–80.1)	(0.024–0.059)	(0.054–0.110)	(0.38–1.10)	(6.10–14.70)
	9 (treated)	79.7	0.023	0.059	0.98	10.40
		(32.1–137.0)	(0.013–0.047)	(0–0.180)	(0.61–1.87)	(6.30–20.20)
Acute leukemia	3 (untreated)	104.3	0.022	0.033	0.78	13.00
		(87.7–127.0)	(0.015–0.031)	(0.020–0.060)	(0.34–1.04)	(10.10–71.20)
	6 (treated)	106.0	0.029	0.037	0.62	13.00
		(81.0–135.0)	(0.023–0.044)	(0–0.077)	(0.48–0.94)	(7.20–18.70)
Chronic myelogenous leukemia	1 (untreated)	91.0	0.026	0.130	0.22	11.50
	4 (treated)	77.6	0.023	0.049	0.63	10.69
		(70.8–95.5)	(0.014–0.035)	(0–0.110)	(0.51–0.83)	(8.20–15.60)
Chronic lymphocytic leukemia	2 (untreated)	62.5	0.033	0.075	0.83	15.20
		(33.1–91.9)	(0.027–0.040)	(0.020–0.130)	(0.80–0.85)	(12.00–18.50)
	4 (treated)	102.5	0.018	0.032	1.09	9.80
		(57.2–125.2)	(0.013–0.024)	(0–0.075)	(0.70–1.80)	(6.90–13.80)
Hodgkins disease	1 (untreated)	77.2	0.022	0.020	0.79	9.70
	10 (treated)	82.1	0.024	0.028	1.03	9.84
		(40.3–141.0)	(0.017–0.031)	(0–0.085)	(0.40–2.50)	(6.20–14.20)
Lymphosarcoma	2 (untreated)	83.8	0.015	0.040	0.90	6.45
		(76.9–90.8)	(0.014–0.015)	(0.020–0.060)	(0.66–1.14)	(5.30–7.60)
	14 (treated)	70.2	0.025	0.043	1.00	10.50
		(31.2–120.8)	(0.013–0.035)	(0–0.140)	(0.36–2.20)	(3.20–15.20)
Multiple myeloma	5 (untreated)	82.9	0.025	0.067	1.11	10.10
		(38.8–121.0)	(0.016–0.030)	(0.020–0.120)	(0.63–1.80)	(5.10–12.30)
	2 (treated)	55.1	0.034	0.075	1.08	14.35
		(37.2–72.9)	(0.034–0.035)	(0.020–0.130)	(0.54–1.60)	(14.30–14.40)

* Mean values and ranges are given in p.p.m. Ranges are in parentheses.

magnesium in the serum to be constantly increased in patients in the anuric state and to correlate well with clouding of the consciousness and prolongation of the Q-T interval of of the electrocardiogram.[16] The levels of magnesium in the serum were found to be elevated in patients with adrenal insufficiency.[17]

For a detailed discussion of disturbances in magnesium metabolism the complete and critical review by Wacker and Vallee is recommended.[5]

We have found no reports of magnesium

abnormalities associated with hematologic disorders.

In none of the diseases studied did we find abnormal levels of magnesium in plasma, except for one patient with untreated Hodgkin's disease whose plasma value was low. (Table I.)

The levels of magnesium in the red blood cells (Table II) were above the normal range in all three patients with myelofibrosis. In patients with acute leukemia, marked elevations were also observed. One of these patients had acute lymphoblastic leukemia. The value for his red blood cells before treatment was high (127 p.p.m.) but returned to normal after one month of treatment with 6-mercaptopurine. Three of four samples from patients with treated chronic lymphocytic leukemia yielded extremely high values. The fourth sample was from a patient who was treated ten years before by radiation with x-rays to the spleen. The value for his red blood cells was normal as in patients with untreated chronic lymphocytic leukemia.

CHROMIUM

Strong evidence exists that chromium is a cancerigenic agent. This is suggested by experiments with rats[18] as well as by epidemiologic studies of workers exposed to high concentrations of chromium compounds in industry.[19–21] It is of particular interest that this malignant influence seems to be chiefly confined in man to the development of bronchogenic carcinoma.[19,20,22–25] The reason for this is unknown.

Ulceration of the skin and nasal septum and a variety of inflammatory conditions of the respiratory tract are common in persons exposed to chromium.[26–28] Contact dermatitis from shoe leather is also an occasional problem.[28] Magnus suggests that chromium enters the epidermis in hexavalent form (which may have direct cytotoxic effects[23]), then is reduced to the trivalent cation which is able to bind protein and thereby induce sensitivity.[29] However, Morris believes that basic chromic sulfate, containing the trivalent cation, is the cause of dermatitis from shoe leather and that in this form chromium (III) may enter the skin directly, being conveyed by perspiration.[28]

We have encountered no reports of studies concerning chromium in the blood or of chromate exposure in patients with hematologic diseases.

In our investigation, values for chromium were found to be consistently normal in both plasma (Table I) and red blood cells (Table II) in all of the diseases studied.

NICKEL

It appears probable that nickel is capable of causing carcinoma of the respiratory tract. Doll[30] estimates the risk of death from cancer of the lung to be five times the normal rate, and from cancer of the nose, 150 times the normal rate, among workers in the nickel refineries in South Wales who were employed prior to 1924. Kincaid et al. believe the responsible agent to be nickel carbonyl.[31]

Nickel carbonyl may cause severe acute intoxication. Immediately after exposure, headache, giddiness, "tightness" in the chest, nausea and weakness may occur, or there may be an asymptomatic period of several days followed by a delayed reaction. This is marked by severe retrosternal pain, dyspnea, weakness and occasional gastrointestinal or genitourinary symptoms. Delirium and convulsions may occur terminally.[32] In fatal cases, death appears to result from severe damage to lung tissue.[33]

The administration of sodium diethyldithiocarbamate to the patient is followed by a marked increase in urinary excretion of nickel which gradually returns to normal, and the patient clinically recovers within two to three weeks.[32] This agent seems to be highly effective therapeutically and probably acts by forming a non-toxic chelate with nickel, which is then rapidly excreted.[34]

The extreme toxicity of nickel carbonyl is recognized by the American Conference of Governmental Industrial Hygienists, which sets the maximum concentration allowable for workmen for an eight hour day at 0.001 p.p.m. in air.[35]

Metallic nickel is important as a cause of contact dermatitis[36] and may cause cross sensitivity with copper.[27] It also has the ability, peculiar among contact allergens, to cause a secondary eruption in areas other than the site of contact.[27,36] Wells believes that sweat may mobilize nickel from metallic contactants, and has demonstrated a peculiar affinity of keratin for this metal. It is his opinion that penetration into the deeper layers of epidermis occurs only at sweat duct and hair follicle openings.[37] Magnus suggests that linkage with soluble proteins may occur in the Malpighian layer.[29]

There are no reports relating nickel to diseases of the blood.

We found the nickel values to be highly variable in the plasma and red blood cells of patients with the diseases which we studied (Tables I and II). We believe that no valid conclusions can be drawn from the data obtained.

COPPER

Deficiency of naturally occurring copper has been observed for some time in ruminants grazing where the forage is poor in copper. Cachexia and poor development of the coat result. In lambs, spastic paralysis has been observed with cerebral demyelination and degeneration of motor tracts in the spinal cord. Deficiency of copper in ruminants has also been found where normal or increased amounts of the metal occur in the herbage; this is presumably due to its presence in an unavailable form.[38]

The occurrence of disease in man due to copper deficiency has not been demonstrated. However, there are many conditions which have been associated with alterations in the levels of copper in the blood. Hypocupremia has been found in patients with the nephrotic syndrome; this is presumably due to the loss of ceruloplasmin through the urine.[39] Hypocupremia has been described as part of a syndrome, occurring in infants, which is characterized by edema, anemia, hypoproteinemia, low levels of iron in the serum and a limited iron-binding capacity. This syndrome has been attributed to a transient unexplained hypercatabolism of protein.[40] A similar condition in infants, thought to be due to copper depletion, has also been reported.[41] Hypocupremia occurs in children with kwashiorkor,[42] sprue[41] and in some infants with iron deficiency anemia.[40] In persons with Wilson's disease, ceruloplasmin is decreased or absent, probably because of a failure in synthesis which is thought to account for the hypocupremia.[43]

Hypocupremia occurs in persons in normal pregnancy and in those with one of a large number of pathologic conditions.[44,45] Treatment with ACTH of patients with acute leukemia resulted in a decline of the levels of copper in plasma which, upon relapse, again became high.[44] The administration of estrogens to thirteen women with gynecologic complaints was followed by an increase in the levels of copper in the serum.[46]

High levels of copper in tissue occur in patients with Wilson's disease in spite of hypocupremia.[43] Koch et al. found normal or only slightly elevated values in most tissues studied which were obtained from eight patients with lymphomas, but the levels in the plasma were high in all of these patients.[47] Toxic and nontoxic goiters have been found to contain increased amounts of copper.[48] In persons with cirrhosis with cholangitis, the liver was found to have a markedly elevated content of copper; this was attributed to failure of excretion by the liver due to biliary obstruction.[49]

The levels of copper in the red blood cells were found to be normal in most of the patients with the conditions studied by Lahey et al. They found the levels to be elevated in only five of seventeen patients with iron deficiency anemia;[44] whereas, Pagliardi et al. observed increased values in all of sixteen patients; these returned to normal upon treatment with iron or blood transfusions. The levels of copper in the red blood cells have been reported to be elevated in patients with a variety of other conditions.[50] Low values for the red blood cells have been determined in persons with carcinomas and hyperthyroidism.[44]

In our study, levels of copper in plasma were found to be elevated in patients with several conditions. Nineteen of twenty-eight samples from patients with Hodgkin's disease and lymphosarcoma showed moderate to marked elevations. These two groups yielded average values that were 50 per cent higher than normal. Only two patients with acute leukemia had extremely high levels and these account for our high average value. One of these returned to normal following treatment of the patient with 6-mercaptopurine. Half the samples from patients with pernicious anemia showed moderately high levels of copper in the plasma; no differences were attributable to treatment with vitamin B_{12}. High levels of copper in the plasma from persons with polycythemia vera were found in only a third of the samples.

Contrary to previous reports,[44,50] we found no elevation of the levels of copper in the red blood cells of six patients with iron deficiency anemia. Samples from the one patient, who was re-examined after treatment with ferrous sulfate at which time his blood count was normal, showed a marked decline in the level of copper in his red blood cells. No consistent abnormality in the amount of copper in the red blood cells was observed.

ZINC

The occurrence of disease due to primary zinc deficiency has not been established in man, however, it is recognized in swine as the cause of parakeratosis. A high intake of calcium aggravates this deficiency.[51] A few balance studies of man have been carried out, but most of the conditions studied are characterized by lowered values for the serum and values for the red blood cells which vary directly with the hematocrit and hemoglobin concentration.[52] In women who had recently delivered, Vikbladh found the levels of zinc in the serum to be slightly lower than normal, but in their newborn infants the values were higher than normal. The values for erythrocytic zinc were slightly elevated in the mothers but markedly reduced in the infants.[53]

Vallee et al. have found a marked decrease in the levels of zinc in the serum of patients with Laennec's cirrhosis. Tissue from the livers of these patients contained subnormal amounts of zinc, but large quantities were excreted in the urine, except in that of one patient who was terminally ill. It is of especial interest that the administration of zinc tended to restore normal excretory patterns.[54,55] These findings suggest zinc depletion but its relation to the pathogenesis of cirrhosis is not yet clear.

Reduction of the levels of zinc in the serum occurs in patients with myocardial infarction and has an approximately inverse relationship to the levels of activity of serum lactic and malic dehydrogenases. It is suggested that this reduction in zinc removes an inhibition, allowing increased activity of these enzymes. This same relationship is present in persons with cirrhosis and pernicious anemia.[56]

Vikbladh observed a decrease in the levels of zinc in the serum of patients with several acute febrile conditions. Statistical analysis showed a negative correlation between the serum zinc level and the amount of fever of the patient. Low values for zinc in the serum of patients with malignant tumors, hepatogenic jaundice, chronic polyarthritis and chronic nephritis were also found. The levels of zinc in the serum were normal for persons with afebrile rheumatic fever, acute nephritis, peptic ulcer and diabetes.[53]

Several persons with diseases of the blood and the blood-forming organs have been studied with respect to the content of zinc in the serum. These values were normal for patients with anemia following gastrointestinal bleeding with the exception of those with bleeding from carcinoma of the stomach in which case the levels appeared to be reduced. Eight patients with iron deficiency anemia had normal levels. However, the levels of zinc in the serum were low for all of the fourteen patients with untreated pernicious anemia; they returned to normal levels with therapy through the administration of liver.[53] Vallee and Gibson reported the zinc content of the red cells to be high in persons with pernicious anemia, and to subsequently diminish with treatment; these values were obtained by calculations based on measurements of the levels in plasma and whole blood.[57] Vikbladh observed low levels of zinc in the serum of patients with leukemia, but equivocal results were obtained from those with multiple myelomata and aplastic and hemolytic anemias.[53] The leukocyte content of zinc was found to be about 10 per cent of normal in persons with myelogenous and lymphatic leukemia.[57]

The content of zinc in tissue has been studied best in persons with diseases of the prostate. Low values were obtained for those with prostatic hyperplasia and prostatitis and even lower values were found in tissues from cancerous glands.[58,59] Therapy with stilbestrol lowered the values slightly more. Koch et al. found increased amounts of zinc in the thyroids of patients with papillary adenocarcinomas[60] and normal or slightly elevated values in most tissues of patients with lymphomas.[47]

Intoxication from zinc is rarely encountered. Grant-Frost and Underwood demonstrated that zinc is capable of inducing severe copper deficiency in the rat.[61] Ingestion of zinc compounds has been implicated in instances of acute[62] and chronic[63] poisoning. Zinc chloride is highly toxic on contact with tissue and may cause dermatitis.[64] Inhalation of zinc chloride smoke has resulted in extensive damage to the respiratory tract with a subsequent high rate of mortality.[65] Inhalation of zinc oxide fumes may cause metal-fume fever, a benign systemic disease.[66] Ethylenediamine tetraacetate produces a diuresis of zinc[67] and would probably be useful in the treatment of persons with chronic zinc intoxication.

Our data indicate consistently low values for zinc in the plasma from patients with untreated pernicious anemia (Table I) and are in agreement with those of previous studies of serum from patients with this condition.[53,57] This suggests that the clotting process does not remove zinc. After treatment of the patients with vitamin B_{12}, the level in the plasma were found to be normal or elevated. Examination of data from individual patients suggests a gradual rise in the level of zinc, following treatment, to a supernormal peak at two to three months and a subsequent decline to normal range. From all three patients with untreated acute leukemia, extremely high values for zinc in the plasma were obtained.

In contrast to the finding of Vallee and Gibson,[57] we obtained normal values for zinc in the red blood cells of all of the four patients with untreated pernicious anemia. Following specific therapy, a slight but inconstant rise was observed. We found no consistent abnormalities concerning the content of zinc in the red blood cells (Table II).

COMMENTS

Except for those patients with pernicious anemia, no differences clearly due to treatment were observed in the patients with these hematologic diseases. Therapy included phlebotomy for those with polycythemia vera, 6-mercaptopurine and prednisone for patients with acute leukemia, alkylating agents and irradiation with x-rays for those with lymphomatous diseases, urethane for persons with multiple myeloma and blood transfusions for patients in most of the groups. One patient with aplastic anemia, who had received a total of sixty-nine blood transfusions, had normal values.

In view of the experiment of Grant-Frost and Underwood,[61] our data were examined to see if a reciprocal relationship exists between copper and zinc. The plasma values showed such a relationship in thirty-six of the 111 samples. In this group, the values for either copper or zinc were definitely above the normal mean value while the others were definitely below. In fifty-eight samples, the levels of either copper or zinc or both were close to the mean. In only seventeen patients, were the values for both metals increased or decreased. This reciprocal relationship was more pronounced in the red blood cells as it was observed in forty-two of the 106 samples. In fifty-seven samples, values for either or both copper and zinc were close to the normal mean, and in only seven persons were the levels of both metals increased or decreased.

SUMMARY

Samples of plasma and red blood cells from seventy-eight adult patients with hematologic diseases were analyzed spectrochemically for magnesium, chromium, nickel, copper and zinc. The literature was briefly reviewed and our data compared with those provided in available reports. Our results are tabulated and discussed.

ACKNOWLEDGMENT

We wish to acknowledge the technical assistance of Mrs. Joyce Mahon, Mrs. Florcine Fitzwater, Mrs. Amory Wade and Mr. Robert Wilkerson.

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A.M.A. Arch Pathol
65:600-607 (1958)

Experimental Studies in Metal Cancerigenesis

IX. Pulmonary Lesions in Guinea Pigs and Rats Exposed to Prolonged Inhalation of Powdered Metallic Nickel

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The successful production of sarcomas affecting bones, connective tissue, nerve tissue, and muscle in rats and rabbits after a parenteral administration of metallic nickel was reported in previous communications.[1,2] While these observations established the fact that metallic nickel is a carcinogen for two species of experimental animals, the cancers were obtained by introducing nickel through a route which is not encountered under occupational conditions of exposure to nickel in man. The tumors produced in experimental animals, therefore, differ in histogenetic type and topographical distribution from those observed in man, which are carcinomas involving the nasal cavity, the paranasal sinuses, and the lungs and developing after inhalation of nickel dust, fumes, and vapors.[3-5]

In studies on occupational carcinogenesis it is important for scientific and medicolegal reasons to produce experimentally cancers of the same histologic type and location in animals by duplicating as closely as practicable the exposure conditions prevailing for man. Hence, rather extensive inhalation experiments with powdered metallic nickel supplied by the International Nickel Company were started several years ago simultaneous with the already reported experiments in which nickel was administered by the parenteral route. Rats, guinea pigs, and mice were used. The results of these investigations are presented in this communication.

Submitted for publication Nov. 25, 1957.

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Experimental Procedures

The nickel powder used was obtained by precipitating nickel from nickel carbonyl and consisted of more than 99% of pure nickel. The majority of particles had a diameter of 4μ or less. The powdered metallic nickel was delivered into the dusting chamber in constant and controlled amounts with the aid of a Wright Dust Feed Mechanism, producing a concentration of nickel averaging 15 mg. of nickel per cubic meter of air. The animals were exposed to this atmosphere for 6 hours a day for 4 to 5 days per week for a maximal period of 21 months, at which time all animals used for experimentation had died.

The following types of animals were used: Guinea pigs of the inbred Strain 13, about 3 months old when placed in the dusting chamber, were chosen because they have an average life span exceeding that of other strains. Animals of this particular strain, moreover, had been used in experiments with carcinogenic agents by other investigators.[6,7] Information on the frequency and type of pulmonary tumors in members of this strain under normal and abnormal conditions therefore was available. A total of 42 guinea pigs (32 males, 10 females) was employed. Members of two strains of rats were exposed when from 2 to 3 months old. One hundred rats of the Wistar strain obtained from a commercial breeder evenly divided as to sex and sixty female rats of the Bethesda Black strain made up the test group (total, one hundred sixty rats). Since both strains had been used in other carcinogenic experiments and had been studied in normal control series, adequate information was available as to the occurrence, frequency, topographical distribution, and histologic types of tumors and their precursor conditions for both strains. Similar information was on hand regarding mice of the C57 Black strain, of which 20 female mice about 2 months of age at the start of the experiment were employed. This strain was chosen because the absence of spontaneous lung tumors among its members would have conveyed distinct significance to the appearance of even only a few pulmonary neoplasms in test animals. The only special control group used in this investigation consisted of nine female guinea

TABLE 1.—*Death Distribution of Guinea Pigs, Rats, and Mice Inhaling Metallic Nickel Dust*

	Exposure, Mo.						
	0-6	7-12	13-15	16-18	19-21	22-24	Total
	Deaths, No.						
Guinea pigs	12	7	20	1	2	0	42
Wistar rats	26	38	7	16	11	2	100
Bethesda rats	7	24	26	3	0	0	60
C_{57} Black mice	5	12	3	0	0	0	20

pigs which had been used as breeders and which were from 18 to 30 months old when they were killed.

None of the test animals were killed. All of them died while being exposed to the inhalation of nickel dust. Autopsies were performed on all animals. The histologic examination of the various internal organs and tissues of all animals showing any grossly demonstrable pathologic changes included in some rats and guinea pigs the region of the paranasal sinuses. Sections from all organs and tissues examined were stained with hematoxylin and eosin. Sections of the lungs of some rats were incinerated for the demonstration of nickel deposits.

Postmortem Observations on Experimental Animals

The death distribution of the animals in the various series is presented in Table 1.

1. *Gross Observations*

(*a*) Guinea Pigs: Postmortem examinations on guinea pigs showed that edema, hyperemia, and hemorrhages of the lungs not infrequently associated with localized pneumonic indurations were common pathologic reactions. Livers often were grayish-red to pale-yellow-brown in color in many animals, suggesting the presence of fatty infiltration. The adrenals in one guinea pig were considerably enlarged and dark red. Two of the female guinea pigs had serous ovarian cysts measuring about 2 cm. in diameter.

(*b*) Rats: The most frequent pathologic reactions seen at autopsy in rats were fibrinopurulent pleurisy, acute and chronic circumscribed and diffuse pneumonic lesions often of extensive character, pulmonary congestion and edema, and cystic bronchiectases with cheesy contents. Nodular neoplastic reactions involving the mesenteric and retroperitoneal lymph nodes and forming medullary white and hemorrhagic and necrotic masses up to 5 cm. in diameter were found in five Wistar rats. Similar-appearing nodes located in the liver were present in four additional Wistar rats, while one Wistar rat had an encapsulated subcutaneously located breast tumor in the left axillary region and two Wistar rats had dark red nodular enlarged pituitary glands. Only one of the Bethesda Black rats exhibited a medullary white and hemorrhagic nodular mass involving the ileocecal lymph nodes, while a second rat of this strain had a cysticly distended nodular uterus filled with cheesy necrotic matter.

(*c*) Mice: The necropsies performed on the mice showed the presence of white medullary masses in the mesentery and inguinal lymph nodes measuring up to 2 cm. in diameter in a mouse dying one month after the start of the experiment. Similar neoplastic abdominal nodes were observed in a second mouse which died 12 months after this event. The lungs of the great majority of mice

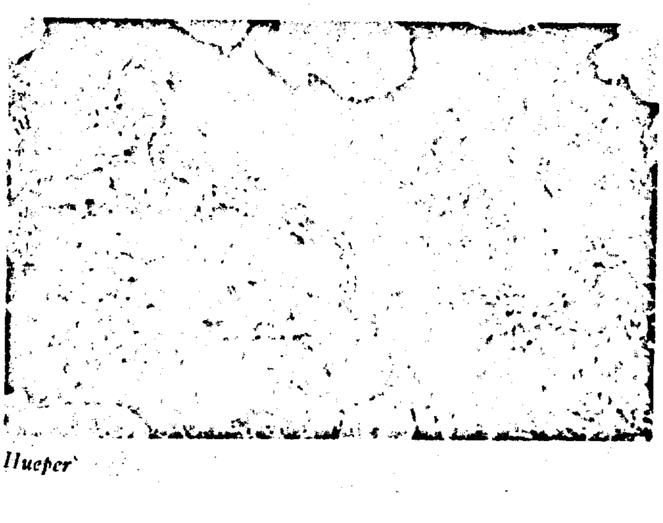

Fig. 1.—Solid epithelial casts of bronchiolar hyperchromatic cells in bronchioli extending into adjacent alveoli; × 143.

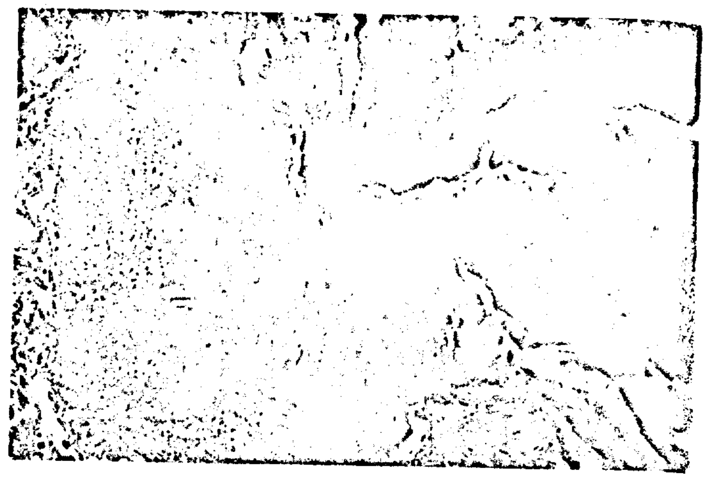

Fig. 2.—Proliferated bronchiolar epithelium filtrating into the wall; × 285.

Fig. 3.—Adenomatoid transformation of peribronchiolar alveoli; × 155.

Fig. 4.—Alveolar adenomatosis involving large areas of the pulmonary parenchyma; × 39.

Fig. 5.—Anaplastic alveolar carcinoma of the lung; × 143.

coming to autopsy were hyperemic and hemorrhagic. The livers were congested.

2. *Histologic Observations*

(*a*) Guinea Pigs: The lungs showed, in addition to frequent local to diffuse edema, hyperemia, and hemorrhages, not infrequently combined with circumscribed areas of leukocytic intra-alveolar infiltrations, the following remarkable reactions in the bronchiolar mucosa and in adjacent alveolar areas. The bronchiolar lumens often were filled in their terminal parts by solid casts of slender oval to spindle-shaped and round hyperchromatic small epithelial cells (Fig. 1). Occasionally similar epithelial proliferations were seen in larger bronchi, where the hyperchromatic cells seemed to infiltrate into the bronchial muscular coat (Fig. 2). The proliferating bronchiolar epithelium extended in many areas into the adjacent alveoli, which were then lined by a cuboidal epithelium and assumed an adenomatoid appearance (Fig. 3). Such areas were often rather large and numerous and were not delineated against the surrounding normal alveoli (Fig. 4). Extensive multicentric adenomatoid formations present in some animals produced the impression of an adenomatosis. In some cases a marked irregularity of the formations and a considerable atypia of the cells in both the bronchiolar and the alveolar formations were noticed. Such lesions resembled, in the bronchioli, carcinomas in situ, in the alveoli, miniature adenocarcinomas. In one animal these changes apparently had progressed sufficiently to form a multicentric anaplastic carcinoma (Figs. 5 and 6). Numerous large

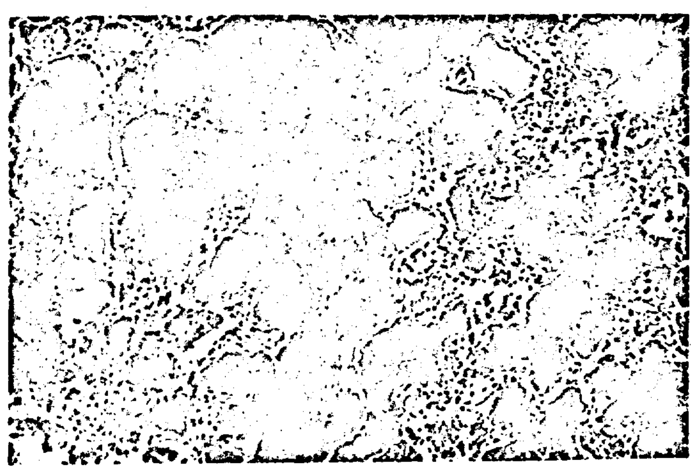

Fig. 6.—Diffuse infiltration of pulmonary alveoli with carcinomatous casts, with partial destruction of intervening pulmonary structures; × 143.

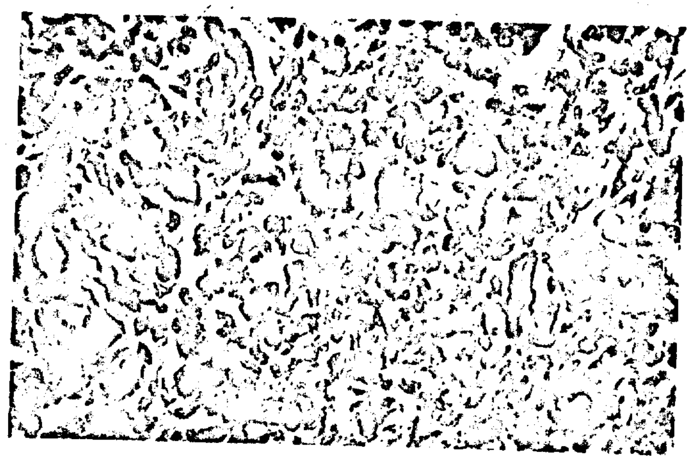

Fig. 7.—Adenomatoid atypical tumor located in the retroperitoneal tissue near the bladder. × 285.

areas in the lung contained alveolar groups solidly filled with small hyperchromatic epithelial cells which invaded the surrounding tissue and extended into the bronchioli. Metastases were not found in the mediastinal lymph nodes or in any other organ or tissue. In a second guinea pig, however, which had numerous adenomatoid formations, a node of adenocarcinomatous tissue was discovered in the lower abdomen near the urinary bladder (Fig. 7). While the primary tumor of the lung was not seen and could have easily been missed at the microscopic examination, since serial sections of the lungs had not been made, there can be little doubt on the basis of its morphology that the abdominal node is of metastatic nature, originating from a pulmonary tumor.

A histologic examination of the region of the nasal sinuses of the guinea pigs was not made, since there was no gross evidence that these structures were abnormal. The particle size of the nickel dust used and the physical form of nickel employed (solid instead of gas or vapor), moreover, did not favor the penetration of nickel into the nasal sinuses. The inadequacy of the available equipment and the general working conditions, on the other hand, prohibited the use of highly toxic nickel carbonyl for chronic experimentation.[15]

In a smaller number of lungs the interalveolar septa and the alveolar lumens were filled with a cellular granulation tissue, often containing giant cells with multiple hyperchromatic nuclei of irregular shape frequently surrounding colorless reflecting rod-shaped inclusions (Fig. 8). The adenomatoid as well as the granulomatous lesions were usually not related to any acute

Fig. 8.—Adenomatoid alveolar area in the lung, with fibrous node containing giant cells attached to the surface of reflecting noncolored rod-like inclusions; × 143.

or chronic inflammatory changes present in the lungs. An analysis of the relation between the period of exposure to nickel dust and the extent of the adenomatoid lesions showed that these increased in frequency, extent, and number with the length of the inhalation period. While a few small peribronchial adenomatoid formations were seen in five of the nine old control guinea pigs, none revealed any diffuse adenomatosis such as that observed in almost all test animals.

The majority of the livers exhibited moderate to marked degrees of vacuolation of the liver cells, which somtimes was associated with moderate periportal fibrosis and round-cell infiltrations. Pericentral hyaline hepatic necroses were present in some animals. Calcium casts were occasionally seen in the medullary tubules of the kidney. The other organs were essentially normal.

(*b*) Rats: The lungs of the rats of both strains exhibited in 15 out of a total of 50 rats studied histologically adenomatoid alveolar formations similar to those seen in the guinea pigs. The epithelial lining of these glandular structures seemed to be extensions from the terminal bronchial mucosa. While in some animals these multicentric lesions were associated with chronic and subchronic inflammatory reactions in the surrounding pulmonary tissue, often they occurred without such an associated pathology and were rather numerous. The paranasal sinuses exhibited, as a rule, chronic inflammatory changes often complicated by mucosal ulcers. The findings of the other organs were not remarkable, with the exception of the occurrence of several tumors. The neoplasms originating from and involving mainly the abdominal and mediastinal lymph nodes were large round-cell sarcomas forming metastases in various organs. One had metastasized into the bone marrow of the sternum. The tumors of the livers were fibrosarcomas or spindle-cell sarcomas. The subcutaneously located neoplasm was an adenofibroma, and the two tumors of the hypophysis were basophil adenomas. Since none of them has any obvious relation to the inhalation of nickel, they will not be further considered.

(*c*) Mice: The lungs were hyperemic and often hemorrhagic. None showed any abnormalities of the bronchial mucosa or any adenomatoid formations. The tumors present in the lymph nodes of two mice were lymphosarcomas, which in all probability were unrelated to the exposure to nickel. Since malignant lymphomas occur "spontaneously" among animals of this strain, it is unlikely that neoplastic reactions may plausibly be attributed under such circumstances to a tissue which had no direct contact with the chemical administered.

Comment

The observations reported indicate that in almost all guinea pigs and in about 50% of the rats of two strains exposed to prolonged inhalation of finely powdered metallic nickel abnormal multicentric adenomatoid formations affecting the alveolar structures and atypical proliferations of the epithelial lining of the terminal bronchioli were present (Table 2).

The four grades are rather arbitrary estimates of adenomatoid involvement of the lungs based on the number and size of adenomatoid formations present. Such data were available on only 37 of the 42 guinea pigs used in the experiment because the organs of 5 animals were too decomposed for permitting a reliable histologic study. In six guinea pigs the intra-alveolar and intrabronchiolar epithelial proliferations assumed a degree of atypia in circumscribed areas approaching the character of microcarcinomas.

TABLE 2.—*Grades of Adenomatoid Proliferations in Lungs of Guinea Pigs After Different Periods of Exposure to Nickel Dust*

Grade	Exposure, Mo. 1-6	7-21
	Guinea Pigs, No.	
1 & 2	7	9
3 & 4	1	20

In one guinea pig an actual anaplastic intra-alveolar carcinoma was found, and in a second one a retroperitoneal node was discovered which in all probability originated from a pulmonary carcinoma. Similar multiple adenomatoid reactions have been reported in guinea pigs after a long-continued total-body exposure to γ-radiation,[7,8] after an intravenous injection of methylcholanthrene,[6] or subsequent to the injection of a diphtheroid bacillus.[9] It is most probable, therefore, that the pulmonary adenomatoid responses in the guinea pigs and rats recorded, as well as, particularly, their carcinomatous sequelae, provide a new additional demonstration of the carcinogenic action of nickel.[1,2] Since these neoplastic and metaplastic responses were obtained by the respiratory introduction of nickel into the lungs, they duplicate to a high degree the conditions which are associated with the appearance of cancers in various parts of the respiratory tract of workers (nasal cavity, nasal sinus, lung) engaged in the smelting of nickel-copper matte in England and Norway.[3,5,10,11] The conclusion, therefore, seems to be justified that they are, like their human counterparts, reactions of the respiratory tissues to the nickel inhaled, which could be demonstrated in the ashed sections prepared from the lungs of some of the rats studied.

The various histologic changes observed in the lungs of guinea pigs and rats make it likely that most of the hyperplastic and metaplastic epithelial proliferations noted had their origin in the terminal bronchioli from where they extended into the adjacent alveoli. Whether or not all of the alveolar-adenomatoid lesions had this bronchiogenic origin, on the other hand, is uncertain, because they were found in areas in which a direct connection with an altered bronchiolus was not apparent. Since, however, serial sections of the lungs were not prepared, this issue must remain undecided. The adenomatoid pulmonary formations resembled in only a very general way those not infrequently seen in mice. They were not nodular in outline and were never papillary in structure but were similar to those described by Norris.[12] They were, moreover, generally distributed throughout the lungs and did not exhibit any preference to the peripheral portions. Because of the frequent absence of concomitant inflammatory reactions in the lungs, the pulmonary adenomatosis cannot be considered a nonspecific response to chronic inflammatory changes, such as those not infrequently noted in the peribronchial tissue of rats suffering from chronic pneumonitis and purulent bronchiectases.[13,14]

The adenomatoid formations seen in the rats which inhaled nickel dust, on the other hand, were often found in areas which were not involved by chronic peribronchial inflammatory changes and which therefore most likely were related in their genesis to the action of inhaled nickel.

Summary and Conclusions

Practically all guinea pigs and many rats belonging to two strains subjected to long-continued inhalation of finely powdered metallic nickel developed multicentric adenomatoid formations of the alveoli and hyperplastic proliferations of the terminal bronchiolar epithelium.

One guinea pig had an anaplastic intra-alveolar carcinoma, while a second guinea pig, with extensive pulmonary adenomatosis, showed an adenomatoid node resembling in structure the pulmonary lesions in the retroperitoneal space of the lower abdomen.

Both benign and malignant pulmonary neoplastic lesions of guinea pigs and benign hyperplastic adenomatoid proliferations in the lungs of rats are attributable to the nickel dust inhaled and may be considered to be etiologic and organic equivalents of the respiratory neoplastic reactions seen in copper-nickel matte smelter workers.

Nickel has been shown to be a carcinogen to rats, rabbits, and guinea pigs, since it is capable of eliciting sarcomas in rats and rabbits after intraosseous and subcutaneous introduction and of producing adenomatoid, carcinomatoid, and carcinomatous reactions

METAL CANCERIGENESIS

in the lungs of guinea pigs after inhalation of nickel dust.

National Cancer Institute, National Institutes of Health (14).

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UDC 615.916:546.74

ON THE TOXICITY OF SOLUBLE COMPOUNDS OF NICKEL WHEN INTRODUCED INTERNALLY

(Article by A. I. Itskova, N. P. Elakhovskaya, O. V. Kolbasova and T. D. Lychnikova of the Sycin Institute of General and Communal Hygiene, the USSR Academy of Medical Sciences, Moscow: Farmakol. Toksikol., Vol. 32, No. 2, 1969, pp 216-218.)

According to the data of Hmelin, Bolotov and Khlopin (quoted by S. N. Cherkinsky), nickel salts introduced into an organism per os are nontoxic. At the same time, there is the information that when these compounds are introduced parenterally, they exert a toxic influence on an organism. On the basis of research by F. Ya. Berenshtein, N. B. Nasyel'sky, I. A. Myedyanik and co-authors, M. I. Shkol'nik, Caso, and Geras, it is possible to conclude that nickel changes blood creation, the carbohydrate and albumen-forming functions of the liver and the activity of a few enzymes when introduced parenterally in doses of from 1 to 10 mg/kg. In the hygenic respect, the question of the toxicity of soluble nickel compounds is of interest because nickel is found in abnormal amounts in a number of sources of water supply. In some cases this is the result of the introduction of nickel and polymetallic ores into sewage reservoirs during output and processing, in others -- the natural increase in the amount of nickel in the environment in regions rich in nickel.

We ascertained the degree of toxicity of chlorous ($NiCl_2 \cdot 6H_2O$) and sulphate ($NiSO_4 \cdot 7H_2O$) nickel by introducing them per os in aqueous solutions.

Method

The experiments were carried out on white rats with an initial weight of 150-170 g., into which aqueous solutions of nickel salt of varying concentrations were introduced with a probe into the stomach once every 24 hours in a quantity of 1 ml/100 g. In the course of the entire experiment, the animals received dry food briquettes and water supply from a suspended glass water bowl. All doses and concentrations were calculated on ionic nickel.

Results

For the introduction of chlorous nickel, LD_{50} was established at 105 mg/kg for males and at 129 mg/kg for females.

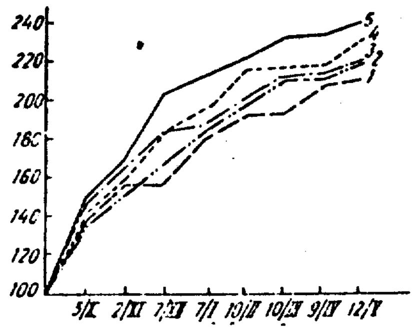
Animals who received nickel in a dose higher than 250 mg/kg died in 5-20 minutes from the moment of priming, exhibiting strongly pronounced adynamia and areflexia. Doses of 172-222 mg/kg produced strong excitement and heightened motor activity immediately after introduction of the solution, which was superseded after 20-40 minutes by a depression of the nervous system. In all the animals we observed edema of the mucous membranes of the mouth and nose, viscous transparent excretions from the mouth cavity, hyperaemia of the nose and cochleae, and epiphora. The mice died 4-7 hours after priming. The same phenomena were observed in animals who received chlorous nickel in a dose of 98-148 mg/kg, but the inhibition stage was superseded by normalization of motor activity. Diarrhea and bloody excretions from the nose appeared in a majority of the rats. Isolated animals died a few days after priming. Rats, into whom chlorous nickel was introduced in a dose of 49-73 mg/kg exhibited all symptoms of intoxication very weakly, and symptoms of acute intoxication were not noted in rats who received that substance in a dose of 25 mg/kg.

Experiments to study the cumulative properties of nickel were conducted on 50 rats, into whom chlorous nickel was introduced daily for 40 days in a dose of 20 and 5 mg/kg. In 2 experimental groups were 20 animals, and the third group of 10 rats served as the control. For the length of the entire experiment, not one rat died. After finishing the priming, a dose of nickel equal to LD_{50} was introduced into half the animals of each group once a day. After this, two rats died in the group where the animals had received nickel in a dose of 20 mg/kg; out of the number of animals who had received 5 mg/kg -- 1, in the control -- 2 rats. Therefore, the percentage of animals who died was less than 50. These data, and also the absence of death in animals after prolonged introduction of nickel in a dose of 20 and 5 mg/kg, when the total introduced was about 8 x and 2 x the LD_{50}, indicate that nickel does not possess cumulative properties.

Chronic experiments were carried out on 72 rats in the course of 7 months. The daily dose of nickel was established at these: 5.0; 0.5; 0.05; 0.005; and, 0.0005 mg/kg. The dynamics of the weight of the animals; the morphological composition of the blood and the maintenance of hemoglobin; the maintenance of common albumen and the ratio of the albuminous fractions of the blood serum; the maintenance of the sulfhydryl groups in the serum; the activity of the cholinesterase and serum amylase; the enzymes of the small intestines -- alkaline phosphatase and enterokinase; the maintenance of ascorbic acid in the liver and adrenal glands; and, the balance and absorption of calcium, magnesium, nitrogen and phosphorus were studied. At the conclusion of the experiment, pathomorphological investigations of the organs and tissues of the experimental animals were conducted.

The rats who had received nickel did not keep up in weight with the animals in the control group (Fig. 1). For animals into whom nickel had been introduced in a dose of 5 mg/kg, the difference was statistically significant (P=0.05). In the balanced experiments a reduction in the absorption of calcium and magnesium (Fig. 2) was noted in rats who had received nickel in a dose of 5 mg/kg ($P<0.05$).

Percentage of Weight Increase



Date of Weighing

Fig. 1. The dynamics of the weight of animals during daily introduction per os of chlorous nickel in a dose of 5 (1); 0.5 (2); 0.005 (3) and 0.0005 mg/kg (4) in comparison with the control (5).

Percentage of Absorption of the Element

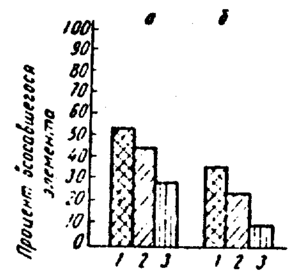


Fig. 2. The absorption of calcium (a) and magnesium (b) in percentages of the introduced dose in the control (1) and during introduction of nickel in a dose of 0.005 (2) and 5 mg/kg (3).

The maintenance of ascorbic acid in the liver was substantially reduced during the introduction of nickel in a dose of 5 mg/kg (up to 5.0 ± 0.86 mg% against 9.1 ± 1.3 mg% in the control, $P<0.05$). Some reduction in the maintenance of ascorbic acid was also noted in the adrenal glands.

The introduction of nickel in a dose of 0.5 mg/kg weight led to a reduction in the activity of alkaline phosphatase and enterokinase in the contents of the intestines, as well as in scrapings of the mucous membrane. This fact produces interest because it is exactly in the upper section of the small intestine that absorption of nickel occurs.

As far as the other tests employed in our research are concerned, no changes were noted.

During pathomorphological investigations of the organs and tissues of the animals, noticeable changes showed up in the mucous membranes of the upper section of the small intestine where an intensified proliferation reaction of the villi of the epithelium and a round-celled infiltration of their stroma was noted. In individual animals the appearances of necrosis of the apical part of the villi were observed. As a result of the introduction of nickel in a dose of 5 mg/kg, extensive proliferation processes of the elements of the lymphoid vessels in the villi's stroma were noted in the small intestine. The appearances of micronecrosis were noted in the apical part of the villi, and here and there were the appearances of atrophy and desquamation of the villi of the epithelium with denudation of the basal membrane.

We did not discover the influence of nickel on the function of blood creation. This influence, noted during parenteral introduction of nickel salt in a dose of 1-10 mg/kg, is, in the opinion of a number of authors, characteristic for nickel and is the basis for comparing its action with the action of cobalt. The absence in our research of changes in the blood creation function can be explained by the fact that during introduction <u>per os</u> up to 90% of the nickel is excreted unabsorbed. Therefore, the absolute quantity of nickel absorbed in an organism is significantly less than that which influences the blood system during parenteral introduction.

Conclusions

1. LD_{50} of nickel for white rats during internal introduction

in aqueous solutions was established at 105 mg/kg for males and 130 mg/kg for females calculated on ionic nickel. Nickel does not possess cumulative properties.

2. During prolonged daily introduction into an organism in a dose of 5 and 0.5 mg/kg, nickel reduces the activity of enzymes -- alkaline phosphatase and enterokinase of the upper section of the small intestine and decreases the absorption of calcium and magnesium. It reduces the maintenance of ascorbic acid in the liver and provokes a lag in the weight of the animals.

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U.DC. · 615 (916) 546 (1974)

УДК 615.916:546.74

О ТОКСИЧНОСТИ РАСТВОРИМЫХ СОЕДИНЕНИЙ НИКЕЛЯ ПРИ ВВЕДЕНИИ ВНУТРЬ

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По данным Гмелина, Болотова, Хлопина (цит. С. Н. Черкинский), соли никеля при введении в организм per os нетоксичны. Вместе с тем имеются сведения, что при парентеральном введении эти соединения оказывают токсическое влияние на организм. На основании исследований Ф. Я. Беренштейна, Н. Б. Насельского, И. А. Медяника с соавторами, М. И. Школьника, Caso, Geras можно заключить, что никель при парентеральном введении в дозах от 1 до 10 мг/кг изменяет кроветворение, углеводную и белковообразующую функции печени и активность некоторых ферментов. В гигиеническом отношении вопрос о токсичности растворимых соединений никеля представляет интерес в связи с тем, что в ряде источников водоснабжения никель содержится в повышенных количествах. В одних случаях это является следствием поступления в водоемы сточных вод предприятий по добыче и переработке никелевых и полиметаллических руд, в других — естественным повышением содержания никеля во внешней среде в районах, богатых никелем.

Мы выяснили степень токсичности хлористого ($NiCl_2 \cdot 6H_2O$) и сернокислого ($NiSO_4 \cdot 7H_2O$) никеля при поступлении per os в водных растворах.

Методика

Опыты проведены на белых крысах с исходным весом 150—170 г, которым водные растворы солей никеля различной концентрации вводили зондом в желудок 1 раз в сутки в количестве 1 мл/100 г. В течение всего опыта животные получали сухой брикетный корм и водопроводную воду из стеклянных навесных поилок. Все дозы и концентрации приведены в расчете на ион никеля.

Результаты

При введении хлористого никеля для самцов LD_{50} составляла 105 мг/кг, а для самок — 129 мг/кг.

Животные, получавшие никель в дозе выше 250 мг/кг, погибали в течение 5—20 мин. от момента затравки при явлениях резко выраженной адинамии и арефлексии. Дозы 172—222 мг/кг сразу же после введения раствора вызывали сильное возбуждение, повышенную двигательную активность, которая через 20—40 мин. сменялась угнетением нервной системы. У всех животных наблюдались отек слизистых оболочек рта и носа, вязкие прозрачные выделения из полости рта, гиперемия носа и ушных раковин, слезотечение. Крысы погибали через 4—7 часов после затравки. У животных, получавших хлористый никель в дозе 98—148 мг/кг, наблюдались те же явления, но стадия торможения сменялась нормализацией двигательной активности. У большинства крыс появлялись кровянистые выделения из носа и понос. Отдельные животные погибали через несколько суток после затравки. У животных, которым хлористый никель вводили в дозе 49—73 мг/кг, все симптомы интоксикации были выражены очень слабо, а у крыс, получавших это вещество в дозе 25 мг/кг, симптомы острой интоксикации не отмечены.

Опыты по изучению кумулятивных свойств никеля проведены на 50 крысах, которым хлористый никель вводили ежедневно в течение

40 дней в дозе 20 и 5 мг/кг. В 2 опытных группах было по 20 животных, 3-я группа из 10 крыс служила контролем. На протяжении всего опыта ни одна крыса не погибла. По окончании затравки половине животных каждой группы была введена однократная доза никеля, равная LD_{50}. После этого в группе, где животные получали никель в дозе 20 мг/кг, погибло 2 крысы; из числа животных, получавших 5 мг/кг,— 1, в контроле — 2 крысы. Таким образом, процент погибших животных был менее 50. Эти данные, а также отсутствие гибели животных при длительном введении никеля в дозе 20 и 5 мг/кг, когда суммарно было введено около 8 и 2 LD_{50}, говорят о том, что никель не обладает кумулятивными свойствами.

Хронические опыты проведены на 72 крысах в течение 7 месяцев. Суточная доза никеля составляла при этом 5; 0,5; 0,05; 0,005; и 0,0005 мг/кг. Изучались динамика веса животных, морфологический состав крови и содержание гемоглобина, содержание общего белка и соотношение белковых фракций сыворотки крови, содержание сульфгидрильных групп в сыворотке, активность холинэстеразы и сывороточной амилазы, ферментов тонкого кишечника — щелочной фосфатазы и энтерокиназы, содержание аскорбиновой кислоты в печени и надпочечниках, баланс и всасывание кальция, магния, азота и фосфора. По окончании опыта проводили патоморфологические исследования органов и тканей подопытных животных.

Крысы, получавшие никель, отставали в весе от животных контрольной группы (рис. 1). Для животных, которым вводили никель в

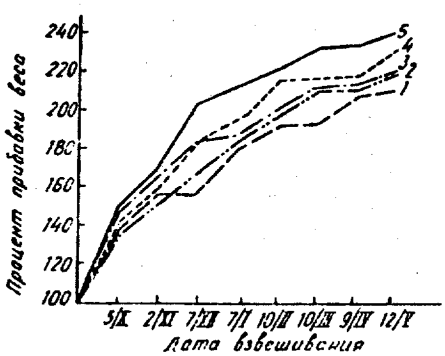

Рис. 1. Динамика веса животных при ежедневном введении per os хлористого никеля в дозе 5 (*1*); 0,5 (*2*); 0,005 (*3*) и 0,0005 *мг/кг* (*4*) по сравнению с контролем (*5*).

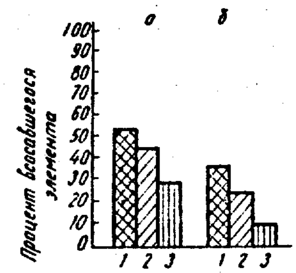

Рис. 2. Всасывание кальция (*а*) и магния (*б*) в процентах от введенной дозы в контроле (*1*) и при введении никеля в дозе 0,005 (*2*) и 5 *мг/кг* (*3*).

дозе 5 мг/кг, разница была статистически достоверна ($P=0,05$). В балансовых опытах было обнаружено снижение всасывания кальция и магния (рис. 2) у крыс, получавших никель в дозе 5 мг/кг ($P<0,05$).

Содержание аскорбиновой кислоты в печени существенно снижалось при введении никеля в дозе 5 мг/кг (до 5,0±0,86 мг% против 9,1±1,3 мг% в контроле, $P<0,05$). Некоторое снижение содержания аскорбиновой кислоты обнаружено и в надпочечниках.

Введение никеля в дозе 0,5 мг/кг веса приводило к снижению активности щелочной фосфатазы и энтерокиназы как в содержимом кишечника, так и в соскобе слизистой оболочки. Этот факт представляет интерес в связи с тем, что именно в верхнем отделе тонкого кишечника происходит всасывание никеля.

По остальным тестам, применявшимся в наших исследованиях, никаких изменений не обнаружено.

При патоморфологических исследованиях органов и тканей животных заметные изменения выявлены в слизистой оболочке верхнего отдела тонкого кишечника, где отмечена усиленная пролиферативная реакция эпителия ворсинок и круглоклеточная инфильтрация их стромы. У отдельных животных наблюдались явления некроза апикальных частей ворсинок. При введении никеля в дозе 5 мг/кг в тонком кишечнике обнаружены обширные пролиферативные процессы лимфоидногистиоцитарных элементов в строме ворсинок. В апикальной части ворсинок отмечены явления микронекроза, местами атрофические явления и десквамация эпителия ворсинок с оголением базальной мембраны.

Нами не обнаружено влияния никеля на функцию кроветворения. Это влияние, отмеченное при парентеральном введении солей никеля в дозе 1—10 мг/кг, является, по мнению ряда авторов, характерным для никеля и дает основание сопоставлять его действие с действием кобальта. Отсутствие в наших исследованиях сдвигов в кроветворной функции может быть объяснено тем, что при введении per os до 90% никеля выводится не всасываясь. Таким образом, абсолютное количество всасавшегося в организм никеля значительно меньше того, которое оказывает влияние на систему крови при парентеральном введении.

Выводы

1. LD_{50} никеля для белых крыс при введении внутрь в водных растворах составляет 105 мг/кг для самцов и 130 мг/кг для самок в расчете на ион никеля. Никель не обладает кумулятивными свойствами.

2. При длительном ежедневном поступлении в организм в дозе 5 и 0,5 мг/кг никель снижает активность ферментов — щелочной фосфатазы и энтерокиназы верхнего отдела тонкого кишечника и уменьшает всасывание кальция и магния. Он снижает содержание аскорбиновой кислоты в печени и вызывает отставание в весе животных.

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Поступила 14/XII 1967 г.

ON THE TOXICITY OF DISSOLUBLE NICKEL COMPOUNDS WITH THEIR ENTERAL INTRODUCTION

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LD_{50} of chlorous and sulfuric nickel administered per os in aqueous solutions constitute when calculated to the value of nickel 105—130 mg/kg. Nickel does not posses cumulative properties and when introduced into the organism daily for a long time in doses of 5 and 0.5 mg/kg it reduces the activity of alkaline phosphatase and enterokinase of small intestine, as well as diminishes absorption of calcium and magnesium. Nickel brings down the level of ascorbic acid in the liver and causes retarded weight gain of the animals.

Biochem J.
35: 877-83 (1941)

97. THE ABSORPTION AND EXCRETION OF 'MINOR' ELEMENTS BY MAN

2. COBALT, NICKEL, TIN AND MANGANESE

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(Received 30 July 1941)

IN a previous paper Kent & McCance [1941] described the results of their work on the absorption and excretion of Ag, Au, Li, B, and V. The experiments now to be described have been carried out in a similar way. As before, the subjects have been either patients or normal persons and the same spectrochemical apparatus and analytical technique have been used. The previous article should be consulted for information about the analytical methods; a full description of the normal subjects and of the metabolic organization has been given by McCance & Widdowson [1941].

Cobalt

Bertrand [1926] and his co-workers showed that Ni and Co were present in all samples of arable soil collected from European countries. They also found these elements in plants and in many human and mammalian organs [Bertrand & Macheboeuf, 1926; Bertrand & Mokragnatz, 1925]. Wohlwill [1907] reported that neither Ni nor Co was absorbed from the gut, but this must have been due to faulty analysis, for absorption has been demonstrated by others [Mascherpa, 1927; Simesen, 1939]. The facility with which 'coast' disease in sheep can be cured by a Co drench [Askew & Dixon, 1936; Wunsch, 1937] and the ease with which polycythaemia can be produced by the oral administration of Co [Josland, 1936] are excellent proofs that at any rate small quantities must be absorbed. If Co has once been absorbed or has been given parenterally, the literature suggests that it is excreted partly by the kidney and partly by the intestine and that the route depends to a large extent upon the nature of the compound and upon how it has been administered. Untersteiner [1931], for example, found that divalent Co was more rapidly eliminated than trivalent Co. Simesen [1939] recovered in the urine of the next 24 hr. 80% of the Co which he had injected as $[Co_3CO(NH_3)_4]Cl$ subcutaneously into rabbits. This compound was excreted unchanged by the kidney. Mascherpa & Perito [1931], who administered $CoCl_2$ to guinea-pigs by the same route, recovered from the urine during the following 10 days less than half the quantity injected. Le Goff [1927] injected 24 mg. of $CoCl_2$ intramuscularly into a man and recovered 6·8 mg. of the salt in the urine within the next 18 hr. He recovered a much smaller quantity (2·64 mg.) from another patient who was a diabetic. Unfortunately the faeces were not examined.

The presence of Co in bile was demonstrated long ago by Stuart [1884]. This was not confirmed by Mascherpa [1927], but has been substantiated by Caujolle [1936] after intravenous administration of the chloride.

The subject of the present experiment was a male hospital patient suffering from carcinoma of the stomach. His kidneys were functioning normally and he did not vomit during the studies. Excluding the preliminary and after periods the experiment lasted 2 weeks. During the first, which served as the control, the patient was given a weighed diet, and urine and faeces were collected quantitatively. During the 2nd week the diet was repeated in every possible respect and urine and faeces were collected as before. On 5 of the 7 days of the 2nd week $CoCl_2$ was injected intravenously and in all 13 mg. of Co were administered in this way. The urine and faeces of both weeks were analysed for Co, but not the food. The results are given in Table 1. The 1st week's data show that the food

Table 1. *The metabolism of cobalt*

Week	Co injected intravenously mg.	Co excreted, mg. Urine	Faeces	Total	Urine Co as % of total
1	0	0·21	1·04	1·25	17
2	13	2·36	1·78	4·14	57
Excess of 2 over 1	13	2·15	0·74	2·89	74

must have contained appreciable amounts of Co and that only 17 % of it passed through the kidney into the urine. During the week in which the injections were given there was a tenfold increase in the urinary excretion and a much smaller increase in the faecal excretion. Assuming the Co intake by mouth to have been the same in both weeks, the results show that of the 13 mg. which were injected intravenously 2·89 were excreted in the week, 74 % of this amount by the kidney. This single experiment confirms in broad outline the results of previous workers on animals and of Le Goff [1927] on man. It suggests that the gut is the main channel of excretion for the Co in natural foods, probably because relatively little is absorbed. Once Co has reached the tissues, however, it indicates that the processes of elimination are very slow, and that the kidney is the organ chiefly responsible. In the rate at which the Co was excreted the present results differ from those of Copp & Greenberg [1941], who administered radioactive Co to two rats and found that 90 % of the Co injected intraperitoneally into one animal was excreted within 4 days. This rapid elimination may be peculiar to the rat.

Nickel

The occurrence and distribution of Ni in soil, plants and in human and animal organs was studied by Bertrand and his fellow workers [Bertrand & Macheboeuf, 1925; 1926; Bertrand, 1926]. They found that Ni and Co had similar distributions in nature, but that animals tended to contain more Co than Ni, plants and soils more Ni than Co [Bertrand & Macheboeuf, 1926]. Mascherpa [1927] and others have studied the absorption and excretion of Ni and its salts. Large doses, whether by mouth or injection, produce a muco-haemorrhagic enteritis, and there is general agreement that this metal is excreted into the intestine rather than by the kidney. This is thought to explain why early workers found that Ni was not absorbed after being taken by mouth. It has been demonstrated in the bile by Stuart [1884], Lehmann [1909] and Caujolle [1937]. Few records have been found of experiments on man, and none of the elimination of Ni after intravenous or subcutaneous administration. A general account of the absorption, excretion and pharmacology of both Ni and Co has been given by Hendrych & Weden [1934].

Two normal men were the subjects for the present studies. Each received daily intravenous injections of $NiCl_2$ during the second week of a long metabolism experiment. The results are shown in Table 2, and it will be seen that before the

Table 2. *The absorption and excretion of nickel*

Subject	Length of period days	Ni intake mg./period		Ni excretion mg./period		Balance mg.	Ni 'recovered' mg.
		Injected	In food	In urine	In faeces		
E. B.	7		2·25	1·67	0·72	− 0·14	
	7	9	1·99	3·42	0·69	+ 6·88	
	7		2·67	3·16	1·19	− 1·68	3·8
N. K.	7		—	2·29	1·20	—	
	7	20	5·51	6·70	1·33	+17·48	
	4		2·21	2·88	0·59	− 1·26	
	7		3·43	3·48	2·11	− 2·16	7·4
	7		3·20	2·61	2·05	− 1·46	

injections were made there was more Ni in the urine than in the faeces. This suggests a reasonably good absorption of the traces of Ni normally present in food. E. B. was almost exactly in balance at this time. N. K.'s balance cannot be given since the figure obtained for his food suggests that it became contaminated with Ni from cutlery during its preparation for analysis. Great precautions were subsequently taken to work only with wooden utensils. During the period of injection the output of Ni rose in the urine of both subjects, so that they eliminated more by this channel than they took in with their food, and they continued to do so in later periods. Clearly Ni was being excreted in the urine. The faecal outputs did not rise during the period of Ni injections, and in subsequent periods they varied in quantity but tended to be slightly higher than they had been during the preliminary week. Only 42 % of the Ni injected into E. B. was recovered and 37 % of that given to N. K. Ni, like Co and Sn (*vide infra*), was excreted slowly and rather incompletely in these experiments and the organ mainly concerned was the kidney, not the gut.

Tin

The metabolism and pharmacology of Sn have been studied spasmodically since foods began to be preserved in cans. Most authors are in agreement that foods may become contaminated with Sn from tinned containers, but small quantities of the metal have also been found in fresh foodstuffs [Boyd & De, 1933; Bertrand & Ciurea, 1931]. Within recent years Sn salts have had a vogue in the treatment of furunculosis and most of the proprietary preparations are intended to be given subcutaneously. Apart, therefore, from the possibilities of industrial poisoning, it is evident that the body is frequently faced with the necessity of having to deal with small amounts of Sn salts. Buchanan & Schryver [1908], using human subjects, and Datta [1940], working with rats, came to the conclusion that tin was poorly absorbed and that small amounts taken by mouth were excreted mainly in the faeces. Sn given by subcutaneous or intravenous injection to animals has been reported to be rapidly removed from the circulation and to be excreted slowly and incompletely by the kidney [Ungar & Bodlander, 1887; Buchanan & Schryver, 1908; Salant *et al.* 1914; 1918; Salant, 1920]. No account has been found of the excretion of Sn after intravenous or subcutaneous administration to man.

Two normal men acted as subjects for the present investigations and each received a total of 28 mg. of Sn as 'Stanoxyl' by daily intravenous injection during the 2nd week of a 21-day metabolism experiment. The 1st week served as a control, during which the absorption and excretion of the Sn present in the food was followed. The results are given in Table 3. Both men were roughly in

Table 3. *The absorption and excretion of tin*

Subject	Length of period days	Sn intake mg./period: Injected during 2nd week	Sn intake mg./period: In food	Sn output mg./period: In urine	Sn output mg./period: In faeces	Balance mg./period	Sn recovered mg.
E. B.	7	0	14·4	7·2	6·6	+ 0·6	
	7	28	13·1	12·6	7·3	+21·2	19·7
	7	0	12·4	13·4	11·9	−12·9	
R. M.	7	0	12·8	8·8	2·9	+ 1·1	16·[illegible]
	14	28	30·5	36·6	10·6	+11·3	

balance during period 1 and were excreting 52 and 75 % of their whole outputs of Sn in the urine. These figures are much higher than those of Datta [1940] or of Buchanan & Schryver [1908]. During subsequent periods the injected Sn was slowly excreted. 70 % of the amount administered was recovered from E. B. and 60 % from R. M. The kidney excreted most of this extra Sn, although the amount in the faeces also rose slightly. The part played by the kidney can be appreciated more fully if the amounts in the food and urine are compared before and after the injections were given.

Manganese

The regular occurrence of Mn in plants and animals—and hence in food—has been a well-established fact for many years [Bertrand, 1939], and many tables have been published showing the usual range of Mn concentration in various foodstuffs [Lindow & Peterson, 1927; Skinner & Peterson, 1928; Davidson, 1929; Remington & Shiver, 1930; Richards, 1930; Peterson & Skinner, 1931]. Perla *et al.* [1939] found that rats might retain little or none of the Mn naturally present in the food, practically all of it passing out in the faeces. When inorganic Mn was added to the food so that the intake was raised from 0·096 to 0·165 and later to 13·45 mg./rat/day, 25 % of the dose was absorbed and retained. Skinner *et al.* [1931] obtained somewhat similar results, but found much higher percentages of Mn normally excreted in the urine.

The kidney has never been found to play an active part in the elimination of Mn [Harnack, 1901]. Normally, only a very small part of the Mn in the food is excreted by this organ, and the figure of 20 %, which Skinner *et al.* [1931] obtained, seems very high in the light of other work. Mn taken by mouth may increase slightly the amount in the urine, but the proportion so excreted always falls [Perla *et al.* 1939; Skinner *et al.* 1931]. Reiman & Minot [1920] showed that these conclusions held good also for human subjects, but in their opinion part at least of the Mn in the faeces represents Mn that has been absorbed and subsequently eliminated. These workers found that after a man had taken 8 g. of franklinite, containing 0·77 g. of Mn, the blood Mn might rise from 0·012 to 0·024 mg./100 ml. within an hour, and that after patients with biliary fistulae had taken 5 g. of franklinite by mouth the Mn in the bile might rise to 10 times its previous level. They held therefore that at least a part of the Mn in the faeces represented metal which had been absorbed, only to be re-excreted. Few references have been found to experiments in which Mn was injected into animals and none to such experiments on man. Cahn [1884] injected toxic doses of Mn salts into rabbits, killed the animals shortly afterwards and analysed their organs. He concluded that Mn so administered was eliminated mainly by the intestine. Quite recently Greenberg & Campbell [1940] have used a radioactive isotope, Mn^{54}, to follow the fate of Mn in the body. 1 mg. of Mn^{54} was injected intraperitoneally into 1 rat and during the subsequent 4 days 90·7 % of it was recovered in the faeces. The result would have been more convincing if the Mn had been given intravenously or subcutaneously rather than intraperitoneally. The quantity excreted in the urine was too small to have any significance and the remaining 9·3 % of the injected Mn^{54} was found in the bodily organs.

One woman and two men—all normal persons—were the subjects of the present experiments. Each received 4–7 injections of Mn butyrate during the 2nd of a 4-period metabolism experiment. E. B. received a diet designed to contain very little Mn; A. M. and P. S. took 40–50 % of their calories in the form of white flour. In other respects the latter's diets were freely chosen. A. M. (the woman), who received 14·3 mg. of Mn, and E. B., who received 19·8 mg., excreted little or none of the injected dose within the time of observation. P. S., however, who received 31·5 mg., probably excreted 16·1 mg. in the faeces during the last 3 weeks of his experiment, but the balances were somewhat irregular and unconvincing. The full results are to be found in Table 4.

Table 4. *The absorption and excretion of manganese*

Subject	Period no. and length	Mn intake mg./period		Mn excretion mg./period		Balance mg./period	Mn recovered mg.
		By mouth	Injected	Urine	Faeces		
A. M.	1 (7 days)	15·5		0·7	13·7	+ 1·1	
	2 (7 days)	19·7	14·4	0·4	17·5	+ 16·1	
	3 (7 days)	23·4		0·3	24·9	− 1·8	3·1
	4 (7 days)	15·6		0·9	18·0	− 3·1	
E. B.	1 (6 days)	10·3		0·3	10·3	− 0·3	
	2 (6 days)	13·4	19·8	0·2	14·5	+ 18·5	
	3 (6 days)	12·5		0·4	12·0	− 0·1	0
	4 (6 days)	14·6		0·4	8·2	+ 6·0	
P. S.	1 (7 days)	46·5		1·2	48·0	− 2·7	
	2 (7 days)	43·5	31·5	0·3	59·0	+ 15·7	
	3 (7 days)	32·4		0·1	37·6	− 5·4	16·1
	4 (7 days)	30·5		0·3	25·1	+ 5·1	

In no subject was there any evidence that any of the injected Mn was excreted in the urine. So far as the urine is concerned these results are essentially in agreement with those of most previous workers. It is evident, however, that Mn is not excreted freely, if at all, by the human bowel when it is injected in the amounts used for these experiments. The partial excretion shown by P. S. may have been the result of the larger doses he received, or of the smaller storage capacity which he possibly possessed. At any rate the difference is thought to have been genuinely one of metabolic behaviour and not to have arisen from contaminations or analytical inconsistencies. The Mn in the foods and faeces of P. S. and in some of the specimens of A. M. was determined chemically by the periodate method of Willard & Greathouse [1917]. The results agreed satisfactorily with those obtained spectrographically, and actually the figures given for P. S. in Table 4 were those obtained by the chemical method.

Table 4 shows that the Mn intakes may vary considerably on natural foods. One of the easiest ways of raising them is to eat a large amount of brown bread, for bran is very rich in Mn. 100 g. of 92 % flour were found to contain 2·15 mg. of Mn and 100 g. of 69 % flour only 0·49 mg. Table 5 shows the balances of 2 persons when they were deriving 40–50 % of their calories from 69 % flour and from 92 % flour.

Table 5. *Manganese intakes and excretions on diets containing large amounts of white and brown flour*

Subject	Diet	Mn intake in food mg./week	Mn excretion, mg./week Urine	Faeces	Total	Balance mg./week
A. M.	White flour	15·5	0·7	13·7	14·4	+1·1
	Brown flour	59·6	0·2	56·2	56·4	+3·2
E. W.	White flour	18·8	0·4	19·2	19·6	−0·8
	Brown flour	61·8	0·6	60·8	61·4	+0·4

Taking the results in Tables 4 and 5 together, they show that the urinary excretion of Mn, like that of Fe, is negligibly small, whatever the intake by mouth or injection. The fact that injecting Mn in these doses did not necessarily provoke any excretion of the metal by either kidney or gut recalls that the human animal has been shown to react to injections of Fe in exactly the same way [McCance & Widdowson, 1938], and illustrates the biochemical and pharmacological affinities of the two metals, some of which were pointed out long ago by Cahn [1884].

Summary

Metabolism experiments on men and women, combined with intravenous injections of Co, Ni, Sn and Mn salts, have shown that

(1) One man excreted about 20% of his food Co in the urine. Injected Co was excreted slowly, mainly by the kidney.

(2) Two men excreted 60–70% of their food Ni in the urine. Injected Ni was excreted slowly, mainly by the kidney.

(3) Two men excreted between 50 and 80% of their food Sn in the urine. Injected Sn was excreted very largely by the kidney.

(4) Only a very small part of the Mn in the food was excreted in the urine, and there was no increase after the intravenous injections of Mn salts. Two persons retained the whole of the injected Mn, a third excreted about 50% by the bowel.

The authors are grateful to Prof. Norrish for placing a spectrograph at their disposal and to Dr W. C. Price for technical advice. The work could never have been undertaken without the help and co-operation of the subjects, and also of Miss B. Alington and Dr E. M. Widdowson.

The greater part of the expenses were covered by a grant made by the Medical Research Council.

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A.M.A. Arch Ind Hyg.
8:48-60 (1953)

NICKEL POISONING

I. Experimental Study of the Effects of Acute and Subacute Exposure to Nickel Carbonyl

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NICKEL carbonyl, $Ni(CO)_4$, has been manufactured since shortly after its discovery by Mond in 1890.[1] Despite its high degree of toxicity, comparatively little trouble has been encountered in manufacturing operations from exposure to the vapor. Until recently there was little use for nickel carbonyl other than for the separation of nickel from its ores. In this process, the nickel carbonyl formed is handled entirely as a vapor in a closed system at atmospheric pressure. Thus, it is not difficult to avoid accidental exposure. On the other hand, the recently revealed utility of nickel carbonyl as a reagent for the synthesis of acrylic esters[2] requires the handling of the pure liquid and its solutions, and thus accidental exposure is much harder to avoid. This suggested the need for an experimental study to determine the $L.D._{50}$ for a number of experimental animals and to discover, if possible, reliable methods of diagnosis and treatment.

GENERAL CONSIDERATIONS OF NICKEL CARBONYL

Properties of Nickel Carbonyl.—The boiling point of nickel carbonyl is 43 C., and its vapor pressure at 25 C. is 380 mm. Hg. This high volatility makes it difficult to avoid exposure by inhalation during handling. In fact, the hazard by inhalation of the vapor is so great in comparison with that due to ingestion or skin absorption of the liquid that only exposure by inhalation was studied in these experiments. The material is unstable, decomposing at an appreciable rate at body temperature in the presence of air and in the absence of a back pressure of carbon monoxide. It is thus probable that the toxic effects of inhaling the vapor are due to its decomposition products rather than to nickel carbonyl as such. The material resulting from decomposition of nickel carbonyl after deposition in the lungs has not been positively identified, but it must be in part an oxidation product of nickel.[3]

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1. Mond, L.; Langer, C., and Quincke, F.: Action of Carbon Monoxide on Nickel, J. Chem. Soc., London, **57**:749-753, 1890.
2. U. S. Patent 2,582,911, dated Jan. 15, 1952, and granted to Harry T. Neher, Edward H. Specht, and Andrew Neuman, Rohm and Haas Company, Philadelphia.
3. Mellor, J. W.: Comprehensive Treatise on Inorganic and Theoretical Chemistry, London, Longmans, Green & Co., 1929, Vol. 5, p. 953.

Literature Values for Acute Toxicity of $Ni(CO)_4$ by Inhalation.—Armit[4] studied the effects of exposure to nickel carbonyl on rabbits, cats, and dogs; Garland[5] studied them on mice, and quite recently Barnes and Denz[6] have reported data on rats and rabbits incidental to their study of dimercaprol (BAL) therapy.

The data contained in these studies are summarized in Table 1. Of these data, only those of Barnes and Denz were obtained with well-controlled flow equipment, and only their data for rats are sufficiently extensive to permit the use of probit analyses for the estimation of $L.D._{50}$. In the other studies the "lethal dose" is not a well-defined quantity, and the data must be regarded as only semiquantitative. Nevertheless, there appeared to be a marked difference in the toxicity of nickel carbonyl for different species.

General Symptoms Reported from Acute Exposure to Nickel Carbonyl.—The symptoms which follow exposure to nickel carbonyl appear to be of two types, immediate and delayed. Even with exposure sufficiently severe to cause death, the initial symptoms are usually mild and not specific, disappearing quickly upon removal

TABLE 1.—*Literature Values for the Lethal Dose of Nickel Carbonyl by Inhalation for Several Species of Experimental Animals*

Animal	Lethal Dose, Mg. per Liter of Air	Time of Exposure, Minutes	Source
Mice	0.1	10	Garland [5]
Rats*	0.4	30	Barnes and Denz [6]
Rabbits	0.3-1.2	30	Barnes and Denz [6]
Rabbits	1.25	65	Armit [4a]
Cats	2.75	90	Armit [4a]
Dogs	2.5	90	Armit [4a]

* The data of Barnes and Denz are given in their publication [6] as the product of concentration and time, with exposure times of 10, 20, and 30 minutes. Their data for rats were converted to the equivalent concentration for 30-minute exposures and plotted on logarithmic probit paper to obtain the value given in the Table for 50% mortality.

of the subject to uncontaminated air. The symptoms usually observed or reported in man include frontal headache, giddiness, nausea, vomiting, and sternal or epigastric pain. In addition, subjects may suffer from mild shock.

The delayed symptoms are usually severer and appear 24 hours or more after the initial symptoms. According to the Medical Department of the Mond Nickel Company,[7] patients suffer from headache, nausea, sleeplessness, and a pain or sense of constriction over the sternum and epigastrium. The pain appears to be aggravated by deep breathing, coughing, or excretion. Cold, clammy skin and cyanotic mucous membranes may be observed. Body temperature seldom rises above 101 F.

4. (*a*) Armit, H. W.: The Toxicology of Nickel Carbonyl, J. Hyg. **7**:525-551, 1907. (*b*) The Toxicology of Nickel Carbonyl, ibid. **8**:565-600, 1908.

5. Garland, G.: The Comparative Toxic Effects of Nickel Carbonyl and Carbon Monoxide, Thesis, University of Maine, 1933.

6. Barnes, J. M., and Denz, F. A.: The Effect of 2-3-Dimercapto-Propanol (BAL) on Experimental Nickel Carbonyl Poisoning, Brit. J. Indust. Med. **8**:117-126, 1951.

7. Frazer, O. B. J., International Nickel Company, New York: Personal communication to the authors.

A survey of the medical literature would indicate that nine fatalities have been reported following exposure to nickel carbonyl.[8] It is possible that there may be duplication in certain of the earlier reports. A description by Armit [4a] of four fatal cases, which corresponds well with those reported later, is quoted.

The symptoms of nickel carbonyl poisoning in man were as follows: immediately after having been exposed to air containing plant gas, there was giddiness and at times dyspnea and vomiting. These symptoms passed off rapidly, as soon as the patients were brought into fresh air. After from 12 to 36 hours, the dyspnoea returned, cyanosis appeared and the temperature began to be raised. Cough with more or less blood-stained sputum occurred on the second day or later. The pulse rate became increased, but not in proportion to the respiratory rate. The heart remained normal. Delirium of varying types frequently occurred, and a variety of other signs of disturbance of the central nervous system was noted. Death took place in the fatal cases between the 4th and 11th days. The chief changes found at post mortem were hemorrhages in the lungs, oedema of the lungs, hemorrhages in the white matter of the brain (in one case this was very extensive), while some doubt exists as to whether any blood changes were present.

According to Brandes,[8a] who reported a fatal case, the characteristic pathologic changes of nickel carbonyl poisoning observed in this case were as follows: (1) hemorrhages into the lung tissue and the white matter of the brain; (2) fatty degeneration of the walls of blood vessels, and (3) edema of the lungs.

In addition to hemorrhages in the lung tissue and brain, hemorrhages have also been found in the adrenals of exposed animals with a greater than 50% frequency.[4a]

Excretion of Nickel Carbonyl in Humans.—According to Drinker and co-workers,[9] nickel is almost never detected in normal urine. The sensitivity of their method is estimated to be less than 0.1 mg. per liter of urine. When normal subjects ingested food cooked in nickel utensils, the highest concentration that was excreted in the urine of their subjects amounted to approximately 4 mg. per liter. Most of the nickel was found to be excreted in the feces. In the stools of subjects eating food cooked in nickel utensils, as much as 30 mg. may be excreted per day, compared with 0.8 mg. per day for normal subjects.

On the other hand, the metabolic studies of Kent and McCance [10] are somewhat at variance with the findings of Drinker and his associates. Kent and McCance found that in the two normal subjects that they studied more nickel was found in the urine than in the feces. The average amounts of nickel excreted in the urine of their subjects were 0.24 and 0.32 mg. per day, respectively; in the feces these amounts were 0.10 and 0.12 mg. per day, respectively. With the assumption of an urinary excretion of 1,500 ml. per day, these values indicate that the concentration

8. (*a*) Brandes, W. W.: Nickel Carbonyl Poisoning: Report of Case, J. A. M. A. **102**: 1204-1206, 1934. (*b*) Hamilton, A., and Johnstone, R. T.: Industrial Toxicology, edited by H. A. Christian, New York, Oxford University Press, 1946, pp. 597-663. (*c*) Mott, F. W.: Carbon Monoxide and Nickel Carbonyl Poisoning: The Systematic Examination of the Cerebral Nervous System in a Case of Poisoning by Illuminating Gas and Two Fatal Cases of Poisoning Occurring in the Carbonyl of Nickel Works, Arch. Neurol., London **3**:246-289, 1907. (*d*) Wales and Western Counties Notes, Lancet **1**:268-269, 1903. (*e*) Bayer, O.: Beitrag zur Toxikologie, Klinik und pathologischen Anatomie der Nickelkarbonylvergiftung, Arch. Gewerbepath. u. Gewerbehyg. **9**:592-606, 1939.

9. Drinker, K. R.; Fairhall, L. T., and Drinker, C. K.: Hygienic Significance of Nickel, J. Indust. Hyg. & Toxicol. **6**:307-356, 1924.

10. Kent, N. L., and McCance, R. A.: Absorption and Excretion of "Minor" Elements by Man: Cobalt, Nickel, Tin and Manganese, Biochem. J. **35**:877-883, 1941.

of nickel is probably less than 0.25 mg. per liter. The values for the concentration of nickel in urine reported by Kent and McCance are higher than those found by Drinker and his associates and by ourselves.

EXPERIMENTAL METHODS

In order to obtain additional data on the toxicity of nickel carbonyl, the effects of exposure were determined on experimental animals. For these studies, albino mice, Wistar strain albino rats, and domestic cats were used.

The method of producing known concentrations of nickel carbonyl in air is illustrated in Figure 1. A solution of nickel carbonyl in absolute alcohol of known concentration (usually within the range 1 to 10%) was introduced into the syringe. The plunger of the syringe was driven forward by the mechanism illustrated to introduce the solution into the air stream at

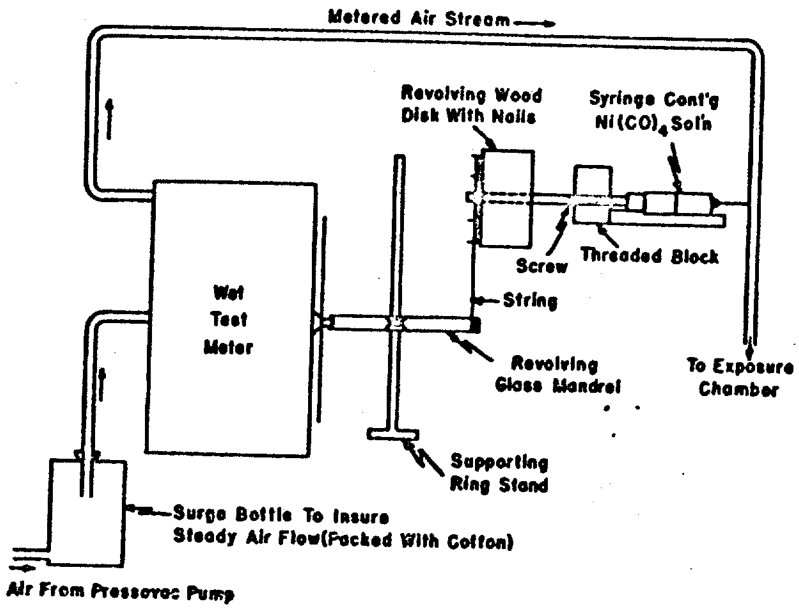

Fig. 1.—Apparatus for preparation of nickel carbonyl-air mixtures.

a predetermined rate. Evaporation was immediate at the low rates of introduction employed. Mandrels of various diameters were used to vary the rate of advance of the screw. This variation combined with the proper choice of concentration of the solution, of the air flow rate, and of the syringe capacity made it possible to obtain concentrations in air ranging from 0.015 to 2.5 mg. of nickel carbonyl per liter of air (2 to 350 ppm by volume). No reduction in meter rate was detected with increasing load. The drive mechanism employed coupled the air stream and the rate of feed by the syringe so that the concentration in the air was unchanged by fluctuation in air supply. The Pressovac pump employed was capable of delivering 1,900 liters of air per hour, and this could be reduced by throttling the intake.

Two exposure chambers were used in our studies. One was an ordinary 25 cm. desiccator, equipped with an inlet reaching to the bottom and with an outlet at the top. The experimental animals were placed on the perforated plate. All mouse exposures and most of the rat exposures were carried out with this chamber. Rates of flow varied from approximately 600 to 1,900 liters per hour. Since the volume of the chamber was 8 liters, turnover times varied from 50 to 15 seconds. Experiments independently carried out showed that nickel carbonyl in air at room temperature decomposed by not more than 5% in 50 seconds. A larger chamber was needed for cat exposures, and a glass box, 45 by 60 by 45 cm., was used initially. The turnover time in this chamber was approximately four minutes. It was first thought that this time was short enough to ensure stability of the nickel carbonyl, but tests later showed that as much as 30% of the material was decomposed on passage through the chamber. Previous exposures in the large chamber were corrected for decomposition, and the volume of the chamber was reduced to eliminate the need for such a correction in future exposures.

Acute and Subacute Toxicity.—In this paper acute toxicity refers to the effects resulting from a single exposure of 30 minutes to nickel carbonyl, whereas subacute toxicity refers to repeated exposures of 30 minutes each at periods a few days apart over several weeks. It might be noted that one cat received six exposures at intervals during the course of four months.

Measurements of L.D.$_{50}$ were made for mice and rats, utilizing the probit method of Miller and Tainter.[11] An insufficient number of cats was exposed to establish a good dosage-probit curve. However, the L.D.$_{50}$ was determined with fair precision by simple inspection of the data. After exposure, the experimental animals were returned to their conventional type cages, and only food and water were made available while awaiting the results of the experiment. In the animals that succumbed, death usually occurred two to three days after exposure.

TABLE 2.—*Estimation of L.D.$_{50}$ for Mice Exposed to Nickel Carbonyl for Thirty Minutes*

Dose, Mg. per Liter	Animals: Exposed	Animals: Dead	Probit
0.0155	12	0	(2.98)
0.0465	15	2	3.89
0.056	10	3	4.48
0.062	29	10	4.60
0.070	20	10	5.00
0.078	22	12	5.11
0.090	10	10	(6.96)

In certain of the studies of both acute and subacute toxicity on rats and cats, determinations were made of the concentrations of hemoglobin and its derivatives, erythrocyte and leucocyte counts, clotting time, prothrombin time, and hematocrit measurements. Prothrombin time was estimated by Quick's method.[12] Spectrophotometric curves were obtained on hemoglobin derivatives by means of a model DU Beckman instrument.

RESULTS

L.D.$_{50}$ for Mice, Rats, and Cats.—L.D.$_{50}$ for Mice: The data for mice are summarized in Table 2.

Intersection of the curve with Probit 5 indicates that the L.D.$_{50}$ is 0.067 mg. per liter (S.E. ±0.003 mg. per liter) for mice exposed for a period of 30 minutes. Our value of 0.067 mg. per liter for 30 minutes of exposure is in reasonable accord with Garland's[5] semiquantitative value of 0.1 mg. per liter for a 10-minute exposure.

L.D.$_{50}$ for Rats: The data for rats presented in Table 3 show that the L.D.$_{50}$ is 0.24 mg. per liter for a 30-minute exposure. This value can be compared with

11. Miller, L. C., and Tainter, M. L.: Estimation of the ED$_{50}$ and Its Error by Means of Logarithmic-Probit Graph Paper, Proc. Soc. Exper. Biol. & Med. **57**:261-264, 1944.

12. Quick, A. J.: Nature of Bleeding in Jaundice, J. A. M. A. **110**:1658-1662, 1938.

the value of 0.4 mg. per liter which we obtained by a similar analysis of the data of Barnes and Denz (Table 1).

In our experiments with mice and rats, the exposed animals were returned to their cages immediately after exposure and were allowed to stay there undisturbed until death or complete recovery occurred. Later experiments were carried out to obtain urine from exposed animals for analysis. One group of six rats were exposed to nickel carbonyl and urine collections were made twice daily thereafter, using Sunderman's technique.[13] All of these animals died—one on the first night, four on the second night, and the sixth on the fourth night. Since the death of only about two of the six rats would have normally been expected, it is believed that the manipulations following exposure were responsible for the increased mortality. These observations were therefore not utilized in the determination of the L.D.$_{50}$ for rats.

TABLE 3.—*Estimation of L.D.$_{50}$ for Rats Exposed to Nickel Carbonyl Vapor for Thirty Minutes*

Dose, Mg. per Liter	Animals		Probit
	Exposed	Dead	
0.17	6	0	(3.27)
0.20	18	9	5.00
0.38	21	17	5.88
0.45	18	15	5.97
0.50	12	12	(6.75)

TABLE 4.—*Exposure of Cats to Nickel Carbonyl Vapor for Thirty Minutes*

Dose, Mg. per Liter	Animals		Time of Death After Exposure, Hr.
	Exposed	Dead	
0.19	1	0	...
0.50	1	0	...
1.24	1	1	216
1.94	2	0	...
2.00	3	3	96, 56, 142
2.11	3	3	96, 36, 72
2.43	1	1	40

L.D.$_{50}$ for Cats: The technique used for exposing cats was similar to that described previously for mice and rats, excepting that the larger exposure chamber was used. Time and facilities did not permit the use of enough cats to obtain the data for a complete dosage-probit curve, but it is believed that the data given in Table 4 permit the choice of an L.D.$_{50}$ with sufficient precision.

Inspection of the data summarized in Table 4 shows that only one animal died after receiving an exposure of less than 2.00 mg. per liter for 30 minutes. Since three animals out of three died after an exposure to 2.00 mg. per liter for 30 minutes and all three cats receiving 2.11 mg. per liter died, it is concluded that the L.D.$_{50}$ for cats must be less than 2.00 mg. per liter. However, both cats exposed to 1.94 mg. per liter survived; hence the L.D.$_{50}$ cannot be much below this figure. A value of 1.9 mg. per liter for a 30-minute exposure is a reasonable choice.

13. Sunderman, F. W.: Method of Collecting Albino Rat Urine, Am. J. Clin. Path., Tech. Sect. 9:11-12, 1945.

Subacute Toxicity.—It is desirable to ascertain whether nickel carbonyl is cumulative under conditions of repeated exposure over periods of a few weeks. The only study of the subacute toxicity of nickel carbonyl published in the literature is that by Garland.[5] In a preliminary study she found that nickel carbonyl is not cumulative and, indeed, that small initial doses build up a tolerance so that doses can be tolerated which would be expected to be lethal if given initially.

Experiments carried out with mice confirmed Garland's findings. A group of five mice was subjected to 10 exposures lasting 30 minutes and covering a period

TABLE 5.—*Multiple Exposures of Mice to Nickel Carbonyl**

Exposure	Dose, Mg. per Liter	Time Between Exposure, Days	Total Time, Days
1	0.016	..	..
2	0.022	7	7
3	0.028	5	12
4	0.038	2	14
5	0.055	2	16
6	0.071	3	19
7	0.098	4	23
8	0.118	4	27
9	0.17	3	30
10	0.19	18	48

* All exposures were for 30 minutes. Five mice were used, and all survived until after the 10th exposure when two died.

TABLE 6.—*Multiple Exposures of Rats to Nickel Carbonyl**

Exposure	Dose, Mg. per Liter	Time Between Exposure, Days	Total Time, Days
1	0.083	..	..
2	0.14	2	2
3	0.20	4	6
4	0.20	3	9
5	0.40	3	12
6	0.40	3	15
7	0.44	4	19
8	0.51	7	26
9	0.52	14	40
10	0.54	8	48

* All exposures were for 30 minutes. One rat out of the group of six died following the 9th exposure; all others survived.

of 48 days. The initial exposure was 0.016 mg. per liter, increasing to 0.19 mg. for the last exposure (Table 5). No deaths occurred until after the 10th exposure, although the 6th and 7th exposures were equal to the L.D.$_{50}$ for mice without previous exposure and the 10th exposure was almost three times this value. The total accumulated exposure was equivalent to 0.76 mg. per liter for 30 minutes, approximately 12 times the L.D.$_{50}$ for mice without previous exposure.

Additional experiments on subacute toxicity (Table 6) carried out with rats led to results similar to those obtained for mice.

Experiments were also carried out with a single cat (Table 7). The cat became noticeably ill after the first exposure, but no symptoms could be observed after the other exposures, in spite of the fact that the sixth was equal to the L.D.$_{50}$ for cats without previous exposure. Severe illness, with an even chance for death, would be expected if this dose were administered to a cat having no previous history of exposure.

These results confirm and extend Garland's preliminary findings that exposure to nickel carbonyl produces a tolerance to it. It is noteworthy that the deaths of mice and rats occurred when the time between exposures was two weeks or more (Tables 5 and 6). It is, therefore, inferred that the increased tolerance resulting from repeated exposures to $Ni(CO)_4$ is of relatively short duration.

Studies on the Blood of Animals Exposed to Nickel Carbonyl.—Carboxyhemoglobin: It has long been known [4a] that the toxic action of nickel carbonyl is not due primarily to its carbon monoxide content but rather to nickel carbonyl itself or to residues resulting from its decomposition. Nevertheless, it was deemed desirable to obtain CO hemoglobin determinations on experimental animals subjected to exposures equal to or exceeding their L.D.$_{50}$. The method of Scholander and Roughton [14] was employed for estimating the CO concentration in the blood. The blood samples were collected under oil by cardiac puncture. Control experiments

TABLE 7.—*Multiple Exposures of a Cat to Nickel Carbonyl**

Exposure	Dose, Mg. per Liter	Total Time After First Exposure, Days
1	0.50	
2	0.64	..
3	1.60	37
4	1.68	67
5	1.24	81
6	1.95	111
		125

* All exposures were for 30 minutes.

carried out with unexposed animals gave blanks averaging about 1% and never exceeding 2.5% saturation. Rats were used for all experiments.

In one experiment, 5% saturation was found in a sample obtained only about 30 seconds after a 10-minute exposure to a concentration of 3 mg. of nickel carbonyl per liter, or about 4 times the L.D.$_{50}$ for acute exposure. The two control animals died within two days. In other experiments no CO hemoglobin was found 30 minutes or more after a 30-minute exposure to a concentration of 1.1 mg. per liter. These findings support the belief that carboxyhemoglobin plays a minor role in the production of the symptoms resulting from nickel carbonyl exposure. Furthermore, the concentrations of carboxyhemoglobin observed are much less than those which would be expected if the carbon monoxide in the nickel carbonyl behaved like uncombined carbon monoxide.[15]

14. Scholander, P. F., and Roughton, F. J. W.: Simple Micro-Gasometric Method of Estimating Carbon Monoxide in Blood, J. Indust. Hyg. & Toxicol. **24**:218-221, 1942.

15. In both cases, 15 to 20% saturation should have been observed according to data obtained with human subjects (Industrial Hygiene and Toxicology, edited by F. A. Patty, New York, Interscience Publishers, Inc., 1949, Chap. 19) if the carbon monoxide had behaved as though it were uncombined. In practice, the absorption rate for rats is probably much greater

(Footnote continued on next page)

Coagulation: Early in the work with nickel carbonyl it was observed that it was much more difficult to withdraw blood from animals suffering from exposure than from controls, and it was suspected that this might be due to a decrease in the clotting time.[17] A group of six rats exposed to 0.27 mg. per liter for 30 minutes was found to have clotting times entirely within the normal range of values when examined two to four hours after exposure and at daily intervals thereafter for a period of four days. It is concluded that clotting time is not affected by exposure.

Prothrombin: Since the prothrombin time is intimately connected with the clotting mechanism, prothrombin times were measured in rats exposed to nickel carbonyl. Preliminary experiments with rats served to establish the technique. Final experiments were conducted with cats. Three cats subjected to a concentration of 1.24 mg. per liter for 30 minutes showed no change in prothrombin time, as compared either with three controls or with their own prothrombin times prior to exposure.

Platelets: It has been shown [18] that the blood platelet count increases sharply after exposure to toxic fumes (such as nitric oxide or phosgene) which produce pulmonary edema. Since nickel carbonyl produces pulmonary edema in experimental animals, a study was made of the blood platelets before and after exposure. No significant change in the number of blood platelets was observed following exposure.

Blood Counts: Erythrocyte and leucocyte counts and estimations of hematocrit percentage and hemoglobin concentrations were obtained in experimental animals before and after exposure. Experiments with rats showed that there were substantial increases in hemoglobin amounting to about 50% above normal at the levels of exposure to nickel carbonyl studied (one-third to three times the $L.D._{50}$ for rats). The erythrocyte counts were closely correlated with the hemoglobin measurements. There did not appear to be a definite correlation of the level of exposure with the increase of the hemoglobin concentration. A slight leucocytosis was observed on the second and third days following exposure.

Abnormal Hemoglobin Pigments: Blood samples taken from cats exposed to nickel carbonyl were examined for the presence of sulfhemoglobin and methemoglobin. None of these pigments or any other hemoglobin derivatives were found on spectroscopic analysis of the blood of exposed rats obtained one hour to one week following exposure.

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than for humans. Our own experiments show that the elimination rate of CO hemoglobin by rats is approximately 10 times greater than that for humans. This difference is presumed to be due to the greater ventilation rate for rats, which would also lead us to expect increased absorption rates. The fact that CO hemoglobin in rats drops to approximately one-fourth its initial value after 30 minutes in fresh air may account for the low value obtained 30 minutes after exposure but not for the value of 5% saturation obtained immediately after a 10-minute exposure to 3 mg. of nickel carbonyl per liter.

16. Footnote deleted.

17. Patek, A. J., Jr., and Stetson, R. P.: Hemophilia: Abnormal Coagulation of Blood and Its Relation to Blood Platelets, J. Clin. Invest. **15**:531-542, 1936.

18. Fleming, A. J.: Method for Handling Cases Gassed with Nitrous Fumes, Including Diagnostic and Prognostic Value of Blood Platelet Counts in Assessing Pulmonary Irritation, Indust. Med. **12**:127-132, 1943.

A survey of the hematologic studies indicated that, with the exception of a moderate hemoconcentration and a slight leucocytosis, there were no other significant changes following exposure.

Nickel Analyses.—Early in the program attempts were made to correlate the concentration of nickel in the urine and the nickel carbonyl exposure, with the objective of establishing a method of diagnosis. Unfortunately, the development of the required analytical method proved to be difficult, owing to the small size of the samples available from the rats being used as experimental animals. Attention was then turned toward the study of the nickel content of urine of a group of persons, using the colorimetric method of analysis described by Alexander, Godar, and Linde.[19] The normal range of values of nickel in urine was found to be from 0.00 to 0.10 mg. per liter. In one case a person accidentally exposed to the vapor of nickel carbonyl yielded 19 mg. of nickel per liter of urine on the day following exposure. During this period, however, this person had received approximately 10 mg. of dimercaprol per kilogram of body weight.

Effect of Dimercaprol on Animals Exposed to Ni(CO)₄.—British antilewisite, 2,3-dimercaptopropanol, (usually called dimercaprol or BAL), developed during

TABLE 8.—*Use of Dimercaprol in Treatment of Nickel Carbonyl Toxicity**

	Control Animals		Treated Animals	
Dose, Mg. per Liter	Exposed	Dead	Exposed	Dead
0.20	18	9	18	0
0.40	9	7	9	3
0.60	9	9	9	8

* All exposures were for 30 minutes. Treatment by intramuscular injection was as follows: day of exposure, 10 mg. of dimercaprol per kilogram of body weight in two doses (0.4 ml. of 0.40% solution of dimercaprol in corn oil); first day after exposure, 8 mg. of dimercaprol per kilogram in two doses; second day after exposure, 3.8 mg. of dimercaprol in two doses of 2.5 and 1.3 mg., and third day after exposure, one dose of 1.3 mg. per kilogram of body weight.

World War II for the treatment of poisoning from nitrogen mustard, is known to combine with certain heavy metals to form stable, nontoxic complexes.[20] Studies undertaken by Braun and associates [20b] showed it to be effective against toxic doses of nickel salts administered by injection. A study was therefore made of the effect of dimercaprol administered to exposed animals.

Rats were used for this study. Three groups receiving different exposures to $Ni(CO)_4$ were each divided into two subgroups, one receiving dimercaprol and the other serving for control purposes. The results, summarized in Table 8, show that dimercaprol increased the $L.D._{50}$ of rats by a factor of approximately 2.

The dosage of dimercaprol given to rats reported in Table 8 is within the range recommended for administration to humans by Eagle and co-workers.[21] Experi-

19. Alexander, O. R.; Godar, E. M., and Linde, N. J.: Spectrophotometric Determination of Traces of Nickel, Indust. & Engin. Chem., Anal. Ed. **18**:206-208, 1946.

20. (*a*) "BAL" (British Anti-Lewisite) in the Treatment of Arsenic and Mercury Poisoning, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **131**:824, 1946. (*b*) Braun, H. A.; Lusky, L. M., and Calvery, H. O.: Efficacy of 2,3-Dimercaptopropanol (BAL) in Therapy of Poisoning by Compounds of Antimony, Bismuth, Chromium, Mercury and Nickel, J. Pharmacol. & Exper. Therap. (Supp.) **87**:119-125, 1946.

21. Council on Pharmacy and Chemistry.[20a] Eagle, H., and Magnuson, H. J.: Systemic Treatment of 227 Cases of Arsenic Poisoning (Encephalitis, Dermatitis, Blood Dyscrasias, Jaundice, Fever) with 2,3-Dimercaptopropanol (BAL), Am. J. Syph. **30**:420-441, 1946.

ments were also carried out with both larger and smaller doses of dimercaprol. Doses three times those for which results are given in Table 8 suggested that, while the L.D.$_{50}$ was slightly increased, the treated animals that died did not live as long as did the controls. Since dosages one-half of those described in Table 8 appeared to be ineffective, the dosages shown in the Table appear to be about optimum.

These findings are to be compared to those of Barnes and Denz,[9] which appeared after our own work with dimercaprol was completed. These investigators studied the use of dimercaprol for rats exposed to nickel carbonyl and concluded that prophylactic administration has some protective value but that therapeutic administration actually increases mortality. It should be noted that their dimercaprol dosage was 60 to 80 mg. per kilogram of body weight given in one or more doses covering a period of not more than four hours following exposure. This dosage is large in comparison with the dosages found by Eagle and Magnuson [21] to produce toxic symptoms. Eagle and Magnuson concluded that doses of 2.5 mg. per kilogram could be employed every four hours with impunity and that doses of 4 mg. are feasible, although they may give rise to some symptoms. However, two-thirds of all subjects showed reaction with doses of 5 mg. per kilogram. We conclude that the diametrically opposed findings of Barnes and Denz and ourselves with respect to the therapeutic value of dimercaprol are due to the differing dosage levels employed. This point of view receives further support from a comparison of the Barnes and Denz data for rats and rabbits. Rabbits were treated with doses of 30 mg. per kilogram given consecutively at 5, 20, 26, 44, and 50 hours after exposure. In contrast to the results noted with rats, the mortality of treated rabbits was less than that of the controls (although the difference was not statistically significant). Our own findings, however, indicate the probability that even the dimercaprol dosages which Barnes and Denz employed for rabbits are larger than optimum.

Pathologic Changes Observed in Exposed Animals.—The most striking gross changes observed at necropsy in experimental animals exposed to nickel carbonyl vapors were those in the lungs. The lungs of the animals that died immediately after exposure showed a severe degree of congestion and pulmonary edema, whereas the lungs of animals that survived for one or more days after exposure usually showed an extensive pneumonitis.

Histologic examinations were made on the organs of rats which had been exposed to concentrations of nickel carbonyl from 0.08 to 2.3 mg. per liter and which were either killed or died from effects of the exposure. The concentrations of the nickel carbonyl vapor and the time following exposure at which death occurred are given in Table 9.

The changes observed in the organs of all these rats were similar; however, the organs from Rat B, which had been exposed to a concentration of 2.3 mg. per liter for 30 minutes and which died 12 hours later, showed the changes to a most conspicuous degree. The findings from this animal will be described briefly, since they are fairly typical of those observed in the other animals.

Lung sections of Rat B showed interstitial pneumonitis, with areas of atelectasis and a few areas of early necrosis. Peribronchial consolidation was present around the larger bronchi. Large amounts of a brownish-black pigment were present throughout the capillaries of the alveolar walls. The pigment was of an amorphous character and was located principally within phagocytic cells, although sharply delineated globules were also found.

Striking changes were also observed in the liver of exposed animals. Degenerative changes occurred in all of the hepatic cells, the cells about the central vein

TABLE 9.—*Rats Exposed to $Ni(CO)_4$ Whose Organs Were Studied Histologically*

Animal	Concentration, Mg. per Liter	Exposure Time, Min.	Time of Death, Hr.	Remarks
Rat A	0.08	30	144	Killed
Rat B	2.3	30	12	Died from effects of exposure
Rat C	2.3	41	41 min.	Died in chamber during exposure
Rat D	0.35	30	15 min.	Killed 15 minutes after exposure

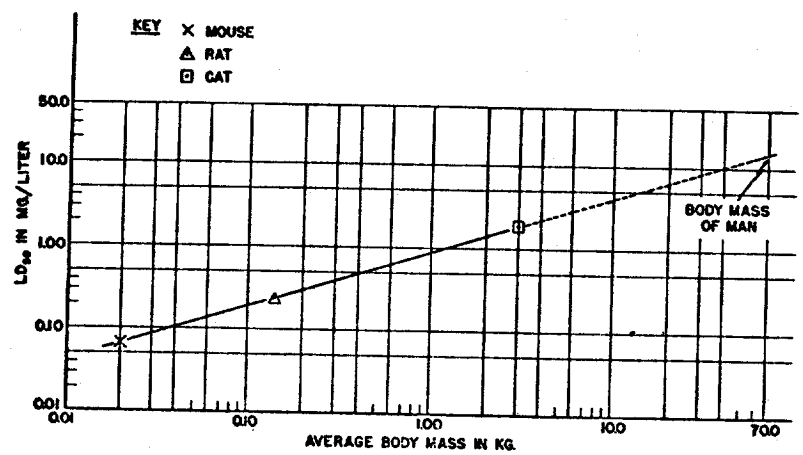

Fig. 2.—Relationship between the $L.D._{50}$ and the size of three experimental animals.

showing almost complete necrosis; toward the periphery of the lobules, the degenerative changes were less marked. Extensive deposits of the brownish-black pigment observed in the lungs were also found throughout the liver.

Sections of the spleen showed the presence of large amounts of pigment, areas of focal necrosis, and degeneration of the reticulum. Large numbers of megakaryocytes were observed.

Degeneration of the tubular epithelium and, to a less extent, of the glomerular tufts occurred in the kidneys. The cells in the vessels lacked their normally clear definition and were agglutinated into amorphous masses. Scattered throughout these masses were nucleated red cells and pigment.

In the pancreas both the acinar cells and islets of Langerhans revealed degenerative changes. In the islet cells there was infiltration, with inflammatory cells that were predominantly eosinophiles.

Sections of the heart muscle were essentially normal.

COMMENT ON DATA FOR MICE, RATS, AND CATS

The large differences in sensitivity to nickel carbonyl of mice, rats, and cats were totally unexpected. Since the $L.D._{50}$ increases sharply with the increasing size of the experimental animal, it might be concluded that man, being much larger than any of the species used, would be comparatively insensitive to the material. In view of its importance, an effort was made to find an equation relating the $L.D._{50}$ for nickel carbonyl to the size or to some other characteristic of the experimental animal. Figure 2 shows a plot on a log-log scale of the $L.D._{50}$ versus the average size of the experimental animal involved. The data lead to the relationship

$$L.D._{50} = 0.009\ (\text{body mass})^{2/3}$$

where the $L.D._{50}$ is the concentration in milligrams of nickel carbonyl per liter of air for a 30-minute exposure and the body mass is expressed in grams. While the equation accurately fits the limited data available, many more points would be required to assess the generality of the relationship and to fix accurately the coefficient and exponent. A similar equation with an exponent of 0.6 is found to represent the variation of oxygen requirement (per unit of mass) for warm-blooded animals versus the mass of the animal. It is thus known that air is inhaled at a faster rate (per unit of mass) by small animals than by larger animals, and this will necessarily lead to a higher toxicity for inhalation poisons for small animals than for larger ones.

It is of interest to note that an extrapolation of Figure 2 leads to an $L.D._{50}$ of 15 mg. per liter for man, assuming a mass of 70 kilograms. This extremely high value for the acute toxicity of nickel carbonyl for man obviously cannot be accepted without confirmation. The result does, however, differ so greatly from the $L.D._{50}$ of nickel carbonyl for mice and rats as to emphasize the necessity of further studies before reliable extrapolation for man can be made from experimental data on inhalation poisons obtained with small experimental animals.

SUMMARY

In anticipation of the increased industrial use of nickel carbonyl, an investigation of its hazards was undertaken.

The $L.D._{50}$ for acute exposure was determined for mice, rats, and cats. The values for each of these species after 30-minute exposures are 0.067, 0.24, and 1.9 mg. per liter, respectively. These findings suggest that the values for $L.D._{50}$ are directly proportional to the ⅔ power of the body masses of the three species studied.

Studies of subacute toxicity indicated that the material is not cumulative over a period of a few weeks and that tolerance develops after exposures to sublethal concentrations.

Therapeutic administration of dimercaprol (BAL) to rats exposed to nickel carbonyl increases their tolerance to nickel carbonyl by a factor of 2.

In humans exposed to nickel carbonyl the amount of nickel excreted in urine is above normal.

Dr. J. F. Woodman, Dr. E. L. Stanley, Mr. Kenneth West, and Dr. Neil Williams assisted with this study.

Effect of Calcium Disodium Ethylenediamine Tetra-Acetate ($CaNa_2$ EDTA) on Experimental Metal Poisoning

IV. Effect of $CaNa_2$ EDTA on Experimental $NiSO_4$ Poisoning

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Source: Arch. intern. pharmacodyn. 120: 80-4, 1959.

In our tests with $CaNa_2$ EDTA, we have decided to also study the effect of $CaNa_2$ EDTA on experimental $NiSO_4$ poisoning. Nickel forms with EDTA *in vitro* a very stable complex, with a log K_{MY} of 18.62, which is included in the series of so-called strong (robust) complexes. As compared to normal complexes, these complexes have a different electron structure, they are sufficiently stable, but they are formed slowly and also dissociate slowly (1). We have studied the toxicity of $NiNa_2$ EDTA, i.e., the toxicity of the complex which we assume is formed in the organism during treatment (therapy) of nickel poisoning with $CaNa_2$ EDTA, and we have also studied the toxicity of $NiSO_4$. We also studied the protective effect of $CaNa_2$ EDTA in the experimental acute poisoning of white mice with $NiSO_4$, as well as the excretion (elimination) of Ni in rats after administration of $NiSO_4$ and $CaNa_2$ EDTA. In our tests, we were mainly concerned in finding out whether the chemical properties of the strong complex could prevent the successful use of $CaNa_2$ EDTA in nickel poisoning.

Method

For our tests, $CaNa_2$ EDTA and $NiNa_2$ EDTA were prepared from Complexon III (Chelaton III, made by the Lachema firm). This $NiNa_2$ EDTA complex was isolated in solid form and its Ni content was determined, which was equal to 9.8%.

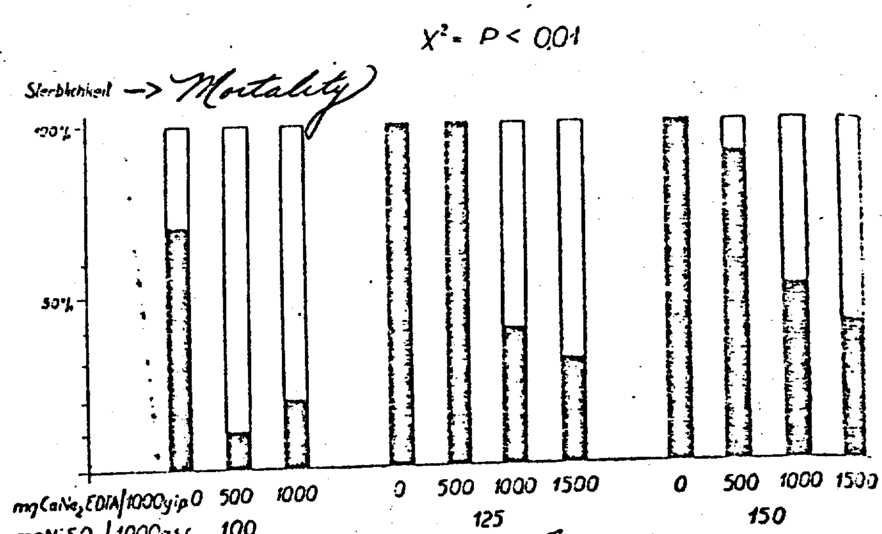
Tests on the excretion of nickel were carried out in metabolic cages, similarly to our previous tests (2, 3). Samples of urine and feces, after dry mineralization in porcelain crucibles, were dissolved in HCl under addition of a 1:1 KNO_3 - Na_2CO_3 mixture and evaporated again. Nickel was determined colorimetrically with dimethylglyoxime (4).

Results

A. In our test on white mice, we determined the toxicity of $NiNa_2$ EDTA. The compound was administered intraperitoneally (i.p.) in a 10% solution; the LD_{50} was 1243.6 mg/kg (i.e. equal to 118.2 mg Ni). The animals were observed for a period of 10 days. Further, we determined the LD_{50} of $NiSO_4$ by i.p. administration of a 10% solution; this value was equal to 37.56 mg/kg (i.e. 7.85 mg Ni).

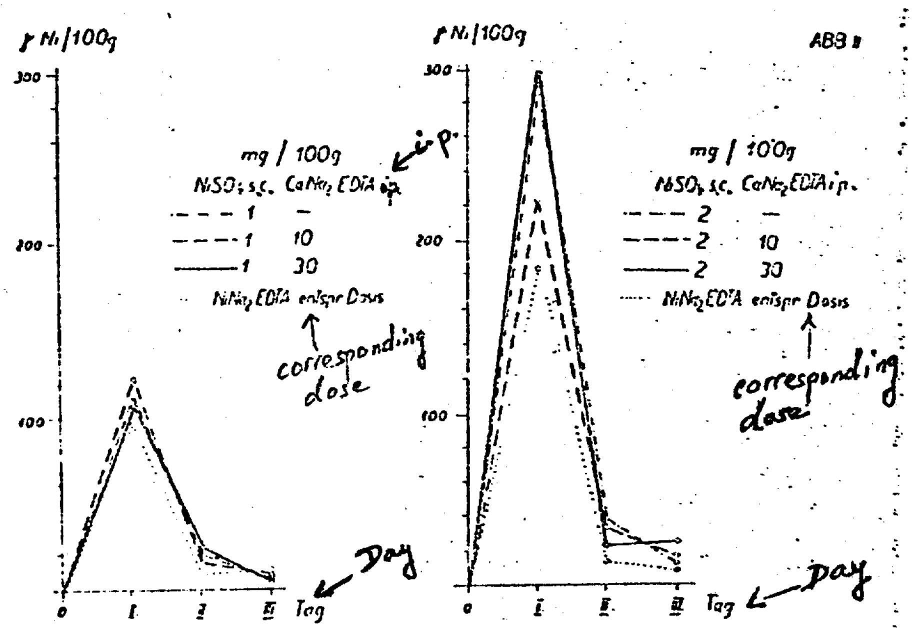
B. The protective effect of $CaNa_2$ EDTA during experimental $NiSO_4$ poisoning was also observed on white mice. $NiSO_4$ was applied in a dose of 100 mg/kg as a 1 $^o/oo$ solution and further in a dose of 125 and 150 mg/kg as a 5 $^o/oo$ solution. $CaNa_2$ EDTA was administered simultaneously i.p. as a 10% solution in doses of 500, 1000, and 1500 mg/kg. The results are illustrated in Fig. 1. In all cases of our test, the protective effect of $CaNa_2$ EDTA proved to be extremely significant ($P < 0.01$).

FIGURE 1



C. Tests on the excretion of $NiSO_4$ were carried out on rats, which were given sublethal doses of $NiSO_4$; namely, once 1 and 2 mg/kg subcutaneously in a 1% solution alone, and another time combined with a simultaneous i.p. administration of 10 and 30 mg $CaNa_2$ EDTA/100 g in a 10% solution. We also applied $NiNa_2$ EDTA in a dose which, as far as the nickel content is concerned, corresponds to a dose of 1 and 2 mg $NiSO_4$/100 g. The excretion of nickel in the urine is shown in Fig. 2.

FIGURE 2



In the first case, since 1 mg $NiSO_4$/100 g was applied, there was no significant difference in the excretion of nickel with the individual combinations ($P > 0.05$). We can see that the major portion of the nickel is excreted during the first 24 hours. Not even in the second case, since 2 mg $NiSO_4$/100 g was administered, did $CaNa_2$ EDTA exert a significant effect on the excretion of nickel in the urine ($P > 0.05$). After administration of the corresponding dose of $NiNa_2$ EDTA we have even noted, in this second case, a significant reduction in the excretion of nickel in the urine ($P < 0.01$).

In the feces, the values of excreted nickel ranged in units of microgram/100 g. After administration of $CaNa_2$ EDTA, no significant changes were noted in the excretion of nickel with feces.

D. In the urine of rats which were given $NiSO_4$ S.C. and $CaNa_2$ EDTA i.p., we were able to determine qualitatively the presence of $NiNa_2$ EDTA and an excess of $CaNa_2$ EDTA.

Free nickel was found only in controls, which were given $NiSO_4$ alone. Free nickel was identified in urine with dimethylglyoxime as a red precipitate. $CaNa_2$ EDTA was detected by the formation of the ruby-red CO_3 complexonate (by reaction with $CO(NO_3)_2$ and oxidation with $K_2Cr_2O_7$). $NiNa_2$ EDTA alone was detected in the urine with the same reagent, after previous addition of NaCN, which is supposed to decompose (breakdown) the Nickel complex. In the urine, $NiNa_2$ EDTA present together with $CaNa_2$ EDTA was determined as follows: the urine sample was divided into 2 equal portions (halves), and to one half of the sample NaCN was added in order to decompose the nickel complex, and then $CO(NO_2)_3$ and $K_2Cr_2O_7$ were added to both halves (portions). The difference in the color intensity corresponds to the amount of $NiNa_2$ EDTA present along with $CaNa_2$ EDTA.

Discussion

M. Rubin (5) states that the toxicity of nickel is reduced by a factor of 70 as a result of the binding of nickel by the EDTA complex. If we compare the LD_{50} of $NiNa_2$ EDTA, i.e., 118.3 mgNi/kg for white mice with the toxicity of $NiSO_4$, i.e., 7.85 mgNi/kg, during i.p. administration, this ratio is equal to 15.

Further, we have found that $CaNa_2$ EDTA exerts a highly significant protective effect when given simultaneously with $NiSO_4$. This problem has already been considered by U. Scudier and V. Tinazzi (b), who studied the effect of $CaNa_2$ EDTA in the experimental poisoning of rabbits by nickel. However, we cannot well compare these tests with ours. The above authors; namely, administered, in the first part of their tests, $NiCl_2$ and $CaNa_2$ EDTA simultaneously by the intravenous route in the same syringe. In doing so, they used the following combinations: 12 mg Ni as metal/kg with 50 and 100 mg/kg of $CaNa_2$ EDTA, and further 15 mg Ni/kg also with 50 and 100 mg/kg of $CaNa_2$ EDTA. However, in order to bind 15 and 12 mg Ni a much larger amount of $CaNa_2$ EDTA than 50 mg is necessary. Actually, Scudier and Tinazzi, in their simultaneous application of both compounds in the above dosage in a single syringe, administered a mixture of $NiNa_2$ EDTA and free $NiCl_2$. Where they administered 15 mg Ni and 12 mg Ni with 100 mg $CaNa_2$ EDTA in a single syringe, they actually gave to the animals a solution of $NiNa_2$ EDTA with a possibly small excess of $CaNa_2$ EDTA. If they had used in all tests at least the theoretical amount of $CaNa_2$ EDTA, which is necessary for binding all Ni, they would have certainly obtained better results. The high value of the LD_{50} of $NiNa_2$ EDTA obtained by us is also a proof of the considerable protective action of $CaNa_2$ EDTA.

Summary

1. In tests on white mice, we were able to establish the extremely high and significant effect of $CaNa_2$ EDTA in the experimental acute $NiSO_4$ poisoning.

2. The LD_{50} of $NiNa_2$ EDTA for white mice was determined, which is equal to 1243.6 mg/kg.

3. In tests on white rats, the effect of $CaNa_2$ EDTA on the excretion of Ni in urine and feces during simultaneous application of $NiSO_4$ s.c. and of $CaNa_2$ EDTA i.p. was noted. $CaNa_2$ EDTA does not increase the amount of metal excreted with the urine.

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Arch. intern. pharmacodyn
120: 80-4 (1959)

DER EINFLUSS VON CA NA_2 EDTA AUF DIE EXPERIMENTELLE METALLVERGIFTUNG

IV. Mitteilung: Der Einfluss von Ca Na_2 EDTA auf die experimentelle Vergiftung mit Ni SO_4

VON

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(Mit tech. Ass. von M. KOSANOVÁ und M. LUCÁKOVÁ)

(Eingegangen am 22-5-1958).

Bei unseren Versuchen mit $CaNa_2EDTA$ haben wir uns entschlossen, auch den Einfluss von $CaNa_2EDTA$ auf die experimentelle Vergiftung mit $NiSO_4$ zu studieren. Nickel bildet mit EDTA in vitro einen sehr festen Komplex, dessen log K_{MY} 18.62 beträgt und der unter die sogenannten robusten Komplexe eingereiht wird. Diese Komplexe haben gegenüber normalen Komplexen eine abweichende Elektronenstruktur, sie sind genügend fest, aber bilden sich langsam und dissoziieren auch langsam (1). Wir haben die Toxizität von $NiNa_2EDTA$ untersucht, d.h. des Komplexes, von dem wir voraussetzen, dass er in dem Organismus bei der Therapie der Nickelvergiftung mittels $CaNa_2EDTA$ gebildet wird, und weiterhin die Toxizität von $NiSO_4$. Wir haben auch den schützenden Einfluss von $CaNa_2EDTA$ bei der experimentellen akuten Vergiftung mit $NiSO_4$ an weissen Mäusen studiert sowie die Ausscheidung von Nickel bei Ratten nach Verabreichung von $NiSO_4$ und $CaNa_2EDTA$. Bei unseren Versuchen ging es uns hauptsächlich darum, ob die chemischen Eigenschaften des robusten Komplexes nicht die erfolgreiche Verwendung von $CaNa_2EDTA$ bei Nickelvergiftung verhindern werden.

Methode

Zu unseren Versuchen haben wir $CaNa_2EDTA$ und $NiNa_2EDTA$ aus dem Komplexon III (Chelaton III — Firma Lachema) bereitet. Dieser $NiNa_2EDTA$-Komplex wurde in fester Form isoliert und auf seinen Nickelgehalt analysiert, der 9.8 % betrug.

Die Versuche hinsichtlich der Ausscheidung von Nickel haben wir ähnlich wie bei den vorangehenden Versuchen in metabolischen Käfigen durchgeführt (2, 3). Die in Porzellantiegeln befindlichen Urin- und Stuhlmuster wurden nach Mineralisierung auf trockenem Wege unter Zusatz eines Gemisches von $KNO_3 + Na_2CO_3$ (1 : 1) in HCL gelöst und wieder verdampft. Das Nickel wurde kolorimetrisch mittels Dimethylglyoxim festgestellt (4).

Ergebnisse

A. Bei dem Versuch an weissen Mäusen haben wir die Toxizität von $NiNa_2EDTA$ festgestellt. Der Stoff wurde in einer 10 %igen Lösung intraperitoneal verabreicht, LD_{50} betrug 1243,6 mg/1 kg (d.i. 118,2 mg Ni). Die Tiere wurden während einer Zeit von 10 Tagen beobachtet.

Wir haben weiters LD_{50} von $NiSO_4$ bei Verabreichung einer 1 %igen Lösung i.p. festgestellt, die 37,56 mg/1 kg (d.i. 7,85 mg Ni) beträgt.

B. Die Schutzwirkung von $CaNa_2EDTA$ bei der experimentellen Vergiftung mit $NiSO_4$ wurde auch an weissen Mäusen beobachtet. Wir haben $NiSO_4$ in einer Dosis von 100 mg/1 kg als 1 ‰ige Lösung appliziert und weiters in einer Dosis von 125 und 150 mg $NiSO_4$/1 kg als ‰ige Lösung. $CaNa_2EDTA$ haben wir gleichzeitig intraperitoneal in einer 10 %igen Lösung in Dosen von 500, 1000 und 1500 mg/1 kg verabreicht. Die Ergebnisse sind in der Abb. 1 veranschaulicht. In allen drei Fällen unseres Versuches erweist sich die Schutzwirkung von $CaNa_2EDTA$ äusserst signifikant ($P < 0{,}01$).

C. Die Versuche mit Ausscheidung von $NiSO_4$ haben wir an Ratten durchgeführt, denen wir subletale Dosen von $NiSO_4$ verabreicht haben, und zwar gaben wir einmal 1 und 2 mg/100 g subkutan in einer 1 %igen Lösung allein, das andere Mal kombiniert mit einer gleichzeitigen intraperitonealen Verabreichung von 10 und 30 mg $CaNa_2EDTA$/100 g in einer 10 % Lösung. Wir haben auch $NiNa_2EDTA$ in einer Dosis appliziert, die, was den Nickelgehalt anbetrifft, einer Dosis von 1 und 2

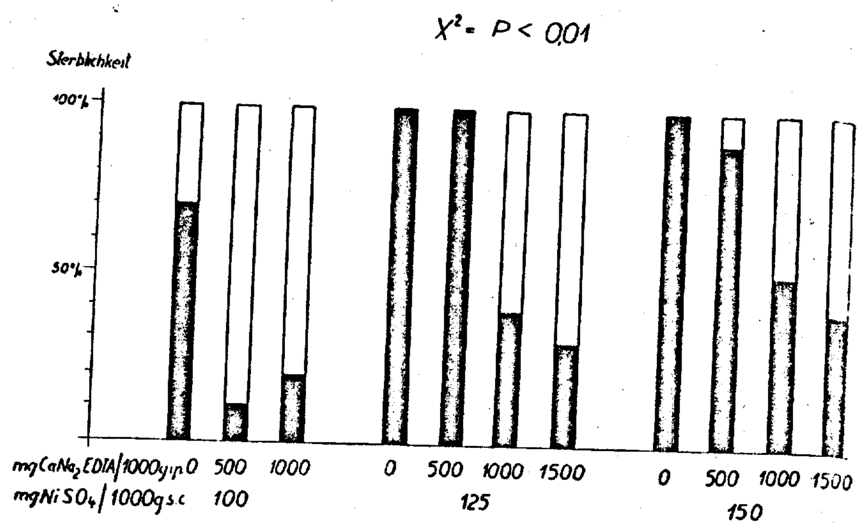

ABB. 1

mg/NiSO$_4$/100 g s.c. entspricht. Die Ausscheidung des Nickels durch den Urin haben wir in Abb. 2 erfasst.

Im ersten Fall, da 1 mg NiSO$_4$/100 g appliziert wurde, zeigt sich überhaupt kein signifikanter Unterschied in der Ausscheidung des Nickels bei den einzelnen Kombinationen (P > 0,05). Wir sehen, dass

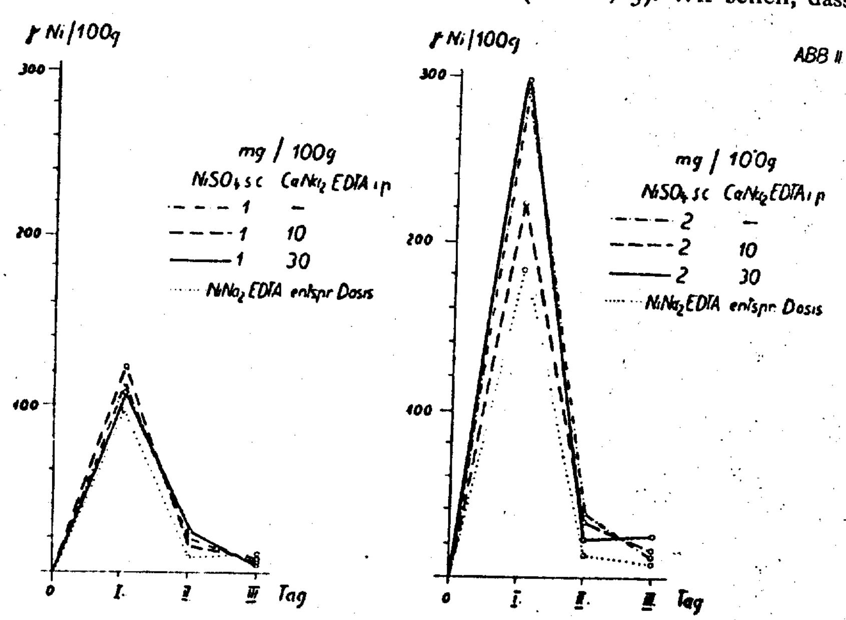

ABB II

der überwiegende Teil des Nickels in den ersten 24 Stunden ausgeschieden wird. Nicht einmal im zweiten Fall, da wir 2 mg $NiSO_4$ 100 g verabreicht hatten, beeinflusste $CaNa_2EDTA$ die Ausscheidung des Nickels im Urin in signifikanter Weise ($P > 0{,}05$). Nach Verabreichung der entsprechenden Dosis von $NiNa_2EDTA$ haben wir in diesem zweiten Fall sogar ein signifikantes Sinken der Ausscheidung des Nickels im Urin verzeichnet ($P < 0{,}01$).

Im Stuhl bewegten sich die Werte des ausgeschiedenen Nickels in den Einheiten von γ/100 g. Nach Verabreichung von $CaNa_2EDTA$ kam es hinsichtlich der Ausscheidung des Nickels durch den Stuhl zu keinen wesentlichen Änderungen.

D. In dem Urin der Ratten, denen wir $NiSO_4$ s.c. und $CaNa_2EDTA$ i.p. verabreicht hatten, stellten wir qualitativ $NiNa_2EDTA$ und einen Überschuss von $CaNa_2EDTA$ fest.

Freies Nickel haben wir bloss bei Kontrollen gefunden, bei denen $NiSO_4$ allein verabreicht worden war. Freies Nickel wurde im Urin durch Dimethylglyoxim als roter Niederschlag nachgewiesen. $CaNa_2$-EDTA wurde durch die Bildung des rubinroten CO_3-Komplexonates nachgewiesen. (Durch Reaktion mit $CO(NO_3)_2$ und Oxydation mit $K_2Cr_2O_7$.). Alleinige $NiNa_2EDTA$ im Urin wurde durch das gleiche Wirkungsmittel nach vorangehendem Zusatz von NaCN, das den Nickelkomplex zerlegen soll, nachgewiesen. Im Urin wurden $NiNa_2EDTA$ nebst $CaNa_2EDTA$ auf folgende Weise festgestellt: Die Urinprobe wurde in 2 Hälften geteilt. Der einen Hälfte der Probe wurde zwecks Zerlegung des Nickelkomplexes NaCN beigefügt und weiter wurden den beiden Hälften $CO(NO_3)_2$ und $K_2Cr_2O_7$ zugesetzt. Der Unterschied in der Färbungsintensität gibt die Menge von $NiNa_2EDTA$ neben $CaNa_2$-EDTA an.

Diskussion

M. Rubin (5) führt an, dass durch Bindung des Nickels an den Komplex mit EDTA die Toxizität des Nickels 70 fach herabgesetzt wird. Vergleichen wir LD_{50} von $NiNa_2EDTA$, d.i. 118,3 mg Ni/1 kg für weisse Mäuse mit der Toxizität von $NiSO_4$, d.i. 7.85 mg Ni/1 kg bei intraperitonealer Applizierung, so ist dieses Verhältniss gleich 15.

Wir haben weiter nachgewiesen, dass $CaNa_2EDTA$ bei gleichzeitiger Verabreichung mit $NiSO_4$ eine hoch signifikante Schutzwirkung hat. Mit dieser Frage haben sich schon auch U. Scudier und V. Tinazzi (6) beschäftigt, die den Einfluss von $CaNa_2EDTA$ bei der experimentellen Vergiftung mit Nickel bei Kaninchen untersucht haben. Doch können wir diese Versuche nicht gut mit den unserigen vergleichen. Sie verabreichten nämlich in dem ersten Teil ihrer Versuche $NiCl_2$ und $CaNa_2EDTA$ gleichzeitig in derselben Spritze intravenös. Sie verwendeten dabei diese Kombinationen : 12 mg Ni als Metall/1 kg mit 50 mg bzw. 100 mg/1 kg $CaNa_2EDTA$, und weiter dann 15 mg Ni/1 kg ebenfalls mit 50 bzw. 100 mg/1 kg $CaNa_2EDTA$. Zur Bindung von 15 und 12 mg Ni ist aber weitaus mehr $CaNa_2EDTA$ als 50 mg notwendig. U. SCUDIER und V. TINAZZI verabreichten bei gleichzeitiger Applizierung beider Stoffe in der angeführten Dosierung in einer Spritze eigentlich ein Gemisch von $NiNa_2EDTA$ und freiem $NiCl_2$. Dort, wo sie 15 mg Ni und 12 mg Ni mit 100 mg $CaNa_2EDTA$ in einer Spritze verabreichten, gaben sie eigentlich eine Lösung von $NiNa_2EDTA$ mit einem event. kleinen Überschuss von $CaNa_2EDTA$. Wenn sie bei allen Versuchen wenigstens die theoretische Menge von $CaNa_2EDTA$, die zur Bindung des gesamten Ni notwendig ist, verwendet hätten, hätten sie bestimmt bessere Ergebnisse erzielt. Der von uns erzielte hohe Wert der LD_{50} von $NiNa_2$-EDTA gibt ebenfalls Zeugniss für die bedeutungsvolle Schutzwirkung von $CaNa_2EDTA$.

ZUSAMMENFASSUNG

1. Bei dem Versuch an weissen Mäusen ist es uns gelungen, die äusserst hohe signifikante Schutzwirkung von $CaNa_2EDTA$ bei der experimentellen akuten Vergiftung mit $NiSO_4$ nachzuweisen.

2. Es wurde LD_{50} von $NiNa_2EDTA$ an weissen Mäusen festgestellt, die 1243,6 mg/1 kg beträgt.

3. Bei den Versuchen an weissen Ratten wurde der Einfluss von $CaNa_2EDTA$ auf die Ausscheidung von Ni im Urin und Stuhl bei gleichzeitiger Applizierung von $NiSO_4$ s.c. und $CaNa_2EDTA$ i.p. beobachtet. $CaNa_2EDTA$ erhöht die im Urin ausgeschiedene Metallmenge nicht.

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Alimentation et la Vie 59(2) 103-153(1971)

Toxic Effects of Certain Trace Elements

VII. Nickel

Nickel is found in its natural state principally in the form of sulfides. Its amount in the earth's crust is on the order of 0.016 p. 100 (Mastromatteo, 1967 [1]).

Its presence in plants was first pointed out by Forchamer in 1855 [2], but it was until the works of Bertrand and his co-workers (1922, 1925, a, b, 1926 [3, 4]) in France, of McHargue (1926 [5]) in the United States and of Berg (1925 [6]) in Germany that its universal distribution in the soil, plants, and animal tissues became known.

These studies showed that nickel is more abundant in soil and plants but much less abundant in animal tissues than cobalt.

A. Biologic Role

In spite of its presence in plants and animal tissues, no scientific work has been able to attribute to it a well defined physiologic role. No diseases caused by its deficiency or overdosage are known. This is fairly surprising because of the similarity of the physical-chemical properties of cobalt and nickel, similarity which could lead to some common physiologic properties. The most recent works show that nickel in the absence of cobalt or in the presence of an adequate quantity of cobalt possesses no therapeutic effect in the treatment of diseases affecting livestock and sheep (Underwood, 1958).

The effect of nickel on different enzymatic systems is well summarized by Royer [8]:

> It is an activator of phosphatases in the spleen and in yeast; of glycerophosphatase of the blood (in the presence of ascorbic acid).
>
> It also activates hepatic arginase, arginase in blood and in brewer's yeast, but the effect of manganese is 10 times more marked.
>
> It would be an inhibitor of intestinal phosphatases; of the lipase of castor oil, of urease and pepsin.
>
> Concerning alcoholic fermentation, small amounts have an activating effect but large doses are inhibiting.
>
> Finally, nickel *in vitro*, activates carboxylase (Speck, 1948, [9]), and trypsin (Sugai, 1944, [10]).
>
> According to Schell (1966, [11]), nickel stabilizes the labile factor intervening in the formation of blood clots.

B. Metabolism

According to Kent and McCance (1941 [12]), an adult absorbs between 0.3 and 0.5 mg of nickel per day.

Green vegetables (lettuce, cabbage, spinach, peas, etc., ...) are relatively rich; 1.5 to 3 ppm. Cereals, potatoes, and fruits are less rich; 0.15 to 0.35 ppm--all these results are given in dry weight (Bertrand *et. al*. [3, 4]).

The preparation and storage of foods, (especially acids) in nickel utensils may lead to the dissolution of significant quantities of this metal (Lehmann, 1908, [13], Normann and Hugel, 1913 [14]).

Milk pasteurized industrially in nickel apparatus contains variable amounts of it. But with the usage in the food industry of new alloys more resistant to corrosion such as monel or iconel, contamination becomes negligeable (Underwood, 1958, [7]).

Administered by the digestive route, nickel salts largely combine with food proteins to form insoluble compounds which are excreted in the feces.

If there is an excess of them, or in the case of hyperacidity of the milieu in the absence of food, they pass into the bloodstream (see Royer, 1952, [8]).

By the parenteral route, the poison is rapidly distributed throughout the organism. The organs which accumulate the most nickel are: the spinal marrow, the brain, the lungs, the heart. The liver and the kidneys contain small quantities (Chittenden and Norris, 1889, [15]).

Riche (1888 [16]) on the other hand, noted significant localization in the liver and kidneys. The recent experiments of Wase et. al. (1954 [17]) with labeled Ni, confirmed these results. The quantities found in the lungs and brain 72 hours after poisoning were of the order, respectively of 38% and 17% of the administered amount.

Further, rapid elimination by the urine and feces was observed. Maximum urinary concentrations were reached 4 hours after the administration of the product.

The percutaneous absorption was nil in animals and in man, even in sensitive subjects (Samitz and Pomerantz, 1958 [18]).

If nickel was given orally, it was eliminated totally or in large part by the feces (Rohde, 1889 [19]; Cannava, 1939 [20]).

Mascherpa (1927 [21]) contended that as opposed to cobalt, it was because of the salts that nickel was excreted. On the other hand, Caujolle (1936 [22]), Kent and McCance (1941 [12]) accorded clear preponderance to urinary elimination.

According to Caujolle (1937 [23]), biliary elimination is always minimal.

C. Normal Amounts

In man, the normal values for nickel in the blood and urine are respectively 3μg/100 ml of blood and 0.76 to 1.1μg per liter of urine (see Browning, 1969 [24]).

However, in cases of myocardial infarction, there is an elevation of the amount of plasmic nickel (D'Alonzo and Pell, 1963 [25]).

The amount of plasmic nickel can thus contribute to the diagnosis of this disease.

Dutoit and Zbinden (1930 [26]) by spectroscopic analysis of organ ashes noted the presence of nickel in the pancreas and in the spleen, and its absence in the liver.

Bertrand and Macheboeuf (1925 b, 1926 [4]), however, gave a value of 0.09 ppm for fresh human liver and only 0.04 ppm for the pancreas.

D. Toxicity of Nickel

We give in Table VII the data concerning the acute toxicity of metallic derivatives of nickel reported in The Handbook of Toxicology [27].

TABLE VII

SEL UTILISÉ 1	VOIE D'ADMINISTRAT. 2	DL mg/kg	ANIMAL
Chlorure de nickel 3 $NiCl_2$, 6 H_2O	I.V.	40-80	chien 4
Sulfate de nickel 5 $NiSO_4$, 6 H_2O	S/C.	62 500-1.000 500-1.000	cobaye 6 lapin 7 chien 4

1--Salt used
2--Route of administration
3--Nickel chloride
4--Dog
5--Nickel sulfate
6--Guinea Pig
7--Rabbit

Orally, high doses of metallic derivatives of nickel cause gastro-intestinal irritation with vomiting and diarrhea. The lethal dose varies according to the species of animals; for the dog, it is on the order of 0.5 g (Greco, 1898 [28]).

By the parenteral route (i.v.), there is observed gastroenteritis undoubtedly due to the excretion of nickel in the intestine, nervous syndromes consisting of trembling, movement similar to Saint-Guy's dance and finally, paralysis. Further, there exists rarely convulsions (especially in the dog).

Death occurs from cardiac failure. The lethal dose of nickel oxide by the intravenous route is 0.01 g/kg in the cat and 0.007 g/kg in the dog (Stuart, 1883 [29]). That of nickel sulfate varies between 0.5 and 1 g for rodents and dogs (Riche, 1888 [30]). For Bertrand and Serbescu (1931 [31]), the amount of 0.10 g/kg was fatal for guinea pigs and rabbits. According to Caujolle and Canal (1939 [32]), dogs succumbed after intravenous injection of a single dose of 0.10 g or 0.20 g of colloidal nickel or nickel chloride.

By the subcutaneous route, lethal doses of the soluble nickel salts are 7 to 8 mg/kg for rabbits and 9 to 16 mg/kg for cats. At autopy, significant changes were observed consisting of edema, hemorrhages and degeneration of the cardiac muscles, the brain, lungs, liver, and kidneys (Armit, 1907, 1908 [33]).

Concerning chronic and semi-chronic poisoning, the few results obtained were very divergent.

Lehmann (1908 [13]) pointed out that cats and dogs tolerate daily doses of metallic nickel salts of 4 to 12 mg/kg during 200 days.

On the other hand, Grushko et. al. (1953 [34]) observed severe alterations of the myocardium and hepatic parenchyma in rabbits receiving nickel sulfate in an average dose of 0.54 mg/kg/day in drinking water for 160 days.

This difference in toxicity would undoubtedly be due to greater absorption of nickel given in drinking water because in food, nickel would exist principally in the form of compounds.

Subjected to inhalation or parenteral administration of metallic nickel during a period of about 6 months, animals often present tumors (Hueper, 1952, 1955, 1958 [35]).

By the parenteral route, Hueper caused bone sarcomas, tumors of the conjunctival and nervous tissue and of the muscles in rats and rabbits. The nasal sinuses and the lungs were not affected.

From inhalation of a concentration of 15 mg/m^3 of metallic nickel dusts of dimensions less than or equal to 4μ during a maximum of 21 months, all the guinea pigs and almost all the rats had tumors, either benign or malignant, in the lungs. Mice, on the other hand, showed no histological alteration in the pulmonary system.

In the guinea pig, the lung showed very clear changes in the form of epithelial proliferations in the bronchial mucosa and extended into several adjacent alveolar zones: some of these lesions resembled bronchial carcinomas and adenosarcomas (in minature) of the alveoli with alveolar anaplastic carcinoma well defined in one animal.

In the rat, adenoid formations were observed in the area of the alveoli and more sarcomas of the abdominal and mediastinal lymphatic modules.

Finally, the effects of nickel on the cardiovascular system, on the respiratory organs, on the digestive system and the related glands, on the nervous system, on the blood, and on the glands of internal secretion are well described by Royer [8].

In man, no systematic poisoning has been found during the course of the utilization of nickel in therapeutics or in the industrial domain.

In therapeutics, nickel was used towards the end of the 19th century as treatment for anemias and as a sedative of the nervous system for head problems, neuralgia and insomnia, either in the form of sulfate or bromide.

With doses higher than the usual therapeutic doses, some vertigo, nausea and vomiting was observed.

In the industrial domain, the metallic derivatives of nickel often cause dermatoses; but the compound responsible for numerous poisonings is nickel carbonyl. The symptomatology and the lesions caused by this derivative are well described in the specialized books on industrial toxicology.

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VOLUME 59 ANNÉE 1971 NUMÉRO 2 (Octobre-Novembre-Décembre)

103-153

BULLETIN
DE LA
SOCIÉTÉ SCIENTIFIQUE D'HYGIÈNE ALIMENTAIRE
DE
L'ASSOCIATION FRANÇAISE DES TECHNICIENS
DE L'ALIMENTATION ANIMALE
ET DE
L'ASSOCIATION FRANÇAISE DE ZOOTECHNIE

L'ALIMENTATION
ET
LA VIE

Publiée sous la direction scientifique
de Lucie RANDOIN de 1942 à 1960

Rédacteur en chef :
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JACQUES LANORE • 12, RUE OUDINOT • PARIS (VII°)
REVUE TRIMESTRIELLE

4 mg/kg pendant 8 jours, le Co provoque d'importantes atteintes cardiaques caractérisées par des œdèmes, des dégénérescences, des séparations de cellules cardiaques, des accumulations de gouttelettes de lipides.

3) *Effets toxiques chez l'homme.*

La plupart des intoxications d'origine non industrielle sont survenues chez l'homme à la suite de l'utilisation du Co contre certaines formes d'anémies. Chez les sujets normaux, l'administration du cobalt se traduit rapidement par une polyglobulie. Dans les différentes formes d'anémies, la réponse est variable : pour les anémies dues à des hémorragies ou à des infections douces, la réponse est bonne ; par contre, pour les anémies macrocytaires ou les infections chroniques sévères, l'effet est moins bon.

Les effets toxiques peuvent se produire même après une dose unique de 500 mg de $CoCl_2$ administré *per os* (WEISSBECKER et MAURER, 1947 [27]) avec des vomissements, des diarrhées et des sensations de chaleur.

Chez l'homme, l'effet immédiat d'une dose suffisamment élevée d'un sel de cobalt hydrosoluble administrée par voie parentérale se traduit, d'après LE GOFF (1929 [30]) par une vasodilatation instantanée des vaisseaux superficiels de la face, des oreilles, de la nuque et du cou, qui dure 5 à 10 minutes, accompagnée d'une accélération cardiaque, d'une chute de la pression sanguine qui s'étend sur une plus grande durée et d'une perception de chaleur assez vive au visage.

On observe, en plus, des respirations lentes, des étourdissements, des palpitations, des surdités nerveuses.

L'administration des doses thérapeutiques journalières de 3 à 4 mg de Co dans le traitement de l'anémie drépanocytaire provoque un effet goitrogène (GROSS et coll., 1955 [46]). La même année, KLINCK (1955 [47]) rapporte 10 cas de goitre observés chez les enfants dont 5 ont reçu des préparations à base de fer et de cobalt. ROBEY et coll. (1956 [48]) signalent un cas d'augmentation de volume de la thyroïde avec tous les symptômes de myxœdème chez un enfant de 17 mois soumis journellement pendant 2 semaines à une préparation à base de fer et de cobalt à la dose de 4 mg de $CoCl_2$ par kg de poids corporel. HOLLY (1955 [49]) par contre, pense que le Co est sans effet sur l'activité de la thyroïde.

ROCHE et LAYRISSE, (1956 [50]) signalent qu'il y a une nette diminution de l'incorporation de l'I^{131} dans les thyroïdes une semaine après l'administration journalière de 150 mg de $CoCl_2$, cependant SCOTT et REILLY (1955 [51]) n'observent aucun changement du métabolisme de l'iode chez l'animal.

Le cobalt peut provoquer encore des intoxications professionnelles très graves avec des dérangements gastriques, des lésions cutanées et des atteintes sévères au niveau pulmonaire (BROWNING, 1969 [52]).

VII. — LE NICKEL.

Le nickel se trouve à l'état naturel principalement sous forme de sulfures. Sa teneur dans l'écorce terrestre est de l'ordre de 0,016 p. 100 (MASTROMATTEO, 1967 [1]).

Sa présence dans les plantes est signalée pour la première fois par FORCHAMMER en 1855 [2], mais il faut attendre les travaux de BERTRAND et coll. (1922, 1925 a, b, 1926 [3, 4]) en France, de Mc HARGUE (1926 [5]) aux Etats-Unis, et de BERG (1925 [6]) en Allemagne pour que sa distribution universelle dans les sols, dans les plantes et dans les tissus animaux soit reconnue.

Ces études montrent que le nickel est plus abondant dans les sols et dans les plantes mais beaucoup plus pauvre dans les tissus animaux que le cobalt.

A — Rôle biologique.

En dépit de sa présence dans les plantes et les tissus animaux, aucun travail scientifique n'a pu lui attribuer un rôle physiologique bien déterminé. On ne connaît pas de maladies dues soit à une carence, soit à un surdosage en nickel. Ceci est assez surprenant, à cause de la similitude des propriétés physico-chimiques du cobalt et du nickel, similitude qui pourrait entraîner quelques propriétés physiologiques communes. Les travaux les plus récents montrent que le nickel en absence du cobalt ou en présence d'une quantité adéquate de cobalt ne possède aucune action thérapeutique dans le traitement des maladies de dépérissement du bétail et du mouton (UNDERWOOD, 1958 [7]).

L'action du nickel sur différents systèmes enzymatiques est bien résumé par ROYER [8] :

— C'est un activateur des phosphatases de la rate et de la levure ; de la glycérophosphatase du sérum (en présence d'acide ascorbique).

— Il active aussi l'arginase hépatique, l'arginase du sérum et de la levure de bière, mais l'action du manganèse est 10 fois plus marquée.

— Il serait inhibiteur des phosphatases intestinales ; de la lipase du ricin, de l'uréase et de la pepsine.

— En ce qui concerne la fermentation alcoolique, les doses faibles ont un effet activant mais les doses fortes sont inhibitrices.

— Enfin, le nickel, *in vitro*, active le carboxylase (SPECK, 1948 [9]) et la trypsine (SUGAI, 1944 [10]).

— D'après SCHELL (1966 [11]) le nickel stabilise le facteur labile intervenant dans la formation du caillot sanguin.

B — Métabolisme.

D'après KENT et Mc CANCE (1941 [12]), un adulte absorbe entre 0,3 et 0,5 mg de nickel par jour.

Les légumes verts (laitue, chou, épinard, pois, etc...) sont relativement riches : 1,5 à 3 ppm. Les céréales, les pommes de terre et les fruits en sont plus pauvres : 0,15 à 0,35 ppm - tous ces résultats sont donnés en poids sec - (BERTRAND et coll. [3, 4]).

La préparation et la conservation des aliments (surtout acides) dans des ustensiles en nickel peuvent entraîner la dissolution des quantités importantes de ce métal (LEHMANN, 1908 [13], NORMANN et HUGEL, 1913 [14]).

Les laits pasteurisés industriellement dans des appareils en nickel contiennent des teneurs variables de celui-ci. Mais avec l'utilisation de nouveaux alliages plus résistants à la corrosion comme le monel ou l'iconel dans l'industrie alimentaire, la contamination devient négligeable (UNDERWOOD, 1958 [7]).

Administrés par voie digestive, les sels de Ni se combinent en grande partie avec les protéines alimentaires pour former des complexes insolubles qui sont excrétés par les fèces. S'ils sont en excès ou en cas d'hyperacidité du milieu et en l'absence d'alimentation, ils passent dans la circulation (voir ROYER, 1952 [8]).

Par voie parentérale, le toxique se distribue rapidement dans tout l'organisme. Les organes qui accumulent le plus de nickel sont : la moelle épinière, le cerveau, le poumon, le cœur. Le foie et le rein contiennent de faibles quantités (CHITTENDEN et NORRIS, 1889 [15]). RICHE (1888 [16]) par contre, signale une localisation importante au niveau du foie et du rein. Les expériences récentes de WASE et coll. (1954 [17]) avec le Ni marqué, confirment ces résultats. Les quantités retrouvées dans les poumons et le cerveau 72 heures après l'intoxication sont respectivement de l'ordre de 38 p. 100 et de 17 p. 100 de la dose administrée. On observe, en plus, une élimination rapide par les urines et par les fèces. Les concentrations urinaires maximales sont atteintes 4 heures après l'administration du produit.

L'absorption percutanée est nulle chez les animaux et chez l'homme même chez les sujets sensibles (SAMITZ et POMERANTZ, 1958 [18]).

Si le nickel est donné par voie orale, il est éliminé en totalité ou en grande partie par les fèces (ROHDE, 1889 [19] ; CANNAVA, 1939 [20]).

MASCHERPA (1927 [21]) soutient même qu'à la différence du cobalt, c'est par les selles que le nickel s'excrète. Par contre, CAUJOLLE (1936 [22]) KENT et Mc CANCE, (1941 [12]) accordent à l'élimination urinaire une nette prépondérance.

D'après CAUJOLLE (1937 [23]) l'élimination biliaire est toujours minime.

C — Teneurs normales.

Chez l'homme, les valeurs normales du nickel dans le sang et dans les urines sont respectivement de 3 μg/100 ml de sang et 0,76 à 1,1 μg par litre d'urine (voir BROWNING, 1969 [24]).

Cependant dans les infarctus du myocarde, il y a une élévation des teneurs du nickel plasmatique (D'ALONZO et PELL, 1963 [25]).

Le dosage du nickel plasmatique peut ainsi contribuer au diagnostic de cette maladie.

DUTOIT et ZBINDEN (1930 [26]) par analyse spectroscopique des cendres d'organes, signalent la présence du nickel dans le pancréas et dans la rate, et son absence dans le foie.

BERTRAND et MACHEBOEUF (1925 b, 1926 [4]) donnent cependant une valeur de 0,09 ppm pour le foie humain frais et seulement 0,04 ppm pour le pancréas.

D — Toxicité du nickel.

Nous donnons dans le *tableau VII* les données concernant la *toxicité aiguë* des dérivés métalliques du nickel, rapportés dans le *Handbook of Toxicology* [27].

TABLEAU VII.

SEL UTILISÉ 1	VOIE D'ADMINISTRAT. 2	DL mg/kg	ANIMAL
Chlorure de nickel $NiCl_2$, 6 H_2O 3	I.V.	40-80	chien 4
Sulfate de nickel $NiSO_4$, 6 H_2O 5	S/C.	62 500-1.000 500-1.000	cobaye 6 lapin 7 chien 8 4

Par voie orale, les doses élevées de dérivés métalliques du nickel provoquent des irritations gastro-intestinales avec des vomissements et des diarrhées. La dose léthale varie selon les espèces animales ; pour le chien elle est de l'ordre de 0,5 g (GRECO, 1898 [28]).

Par voie parentérale (S/C ou I.V.) on observe une gastro-entérite due sans doute à une excrétion du nickel dans l'intestin, des syndromes nerveux consistant en tremblements, en mouvements rappelant la *danse de Saint-Guy* et finalement la paralysie. Il existe en plus, quelques rares convulsions (surtout chez le chien). La mort survient par défaillance cardiaque. La dose léthale de l'oxyde de nickel par voie intraveineuse est de 0,01 g/kg chez le chat et de 0,007 g/kg chez le chien (STUART, 1883 [29]). Celle du sulfate de nickel varie entre 0,5 et 1 g pour les rongeurs et les chiens (RICHE, 1888 [30]). Pour BERTRAND et SERBESCU (1931 [31]) la dose de 0,10 g/kg est mortelle au cobaye et au lapin. D'après CAUJOLLE et CANAL (1939 [32]), les chiens succombent après l'injection intra veineuse d'une dose unique de 0,10 g ou de 0,20 g de nickel colloïdal ou de chlorure de nickel.

Par voie sous-cutanée, les doses léthales des sels solubles de nickel sont de 7 à 8 mg/kg pour les lapins et de 9 à 16 mg/kg pour les chats. A l'autopsie, s'observent de grands changements consistant en œdème, hémorragie et dégénérescence au niveau des muscles cardiaques, du cerveau, du poumon, du foie, du rein (ARMIT, 1907, 1908 [33]).

En ce qui concerne **l'intoxication chronique et semi-chronique,** les quelques résultats obtenus sont très divergents.

LEHMANN (1908 [13]) signale que les chats et les chiens tolèrent des doses journalières de sels métalliques de nickel de 4 à 12 mg/kg pendant 200 jours.

Par contre, GRUSHKO et coll. (1953 [34]) observent des altérations sévères du myocarde et du parenchyme hépatique chez les lapins recevant le sulfate de nickel à la dose moyenne de 0,54 mg/kg/j dans l'eau de boisson pendant 160 jours.

Cette différence de toxicité serait due sans doute à une absorption plus grande du nickel donnée sous forme d'eau de boisson, car dans les aliments le nickel existerait principalement sous forme de complexes.

Soumis à une inhalation ou à une administration parentérale de nickel métallique pendant une période de l'ordre de 6 mois, les animaux présentent souvent des tumeurs (HUEPER, 1952, 1955, 1958 [35]).

Par voie parentérale, HUEPER provoque des sarcomes osseux, des tumeurs des tissus conjonctifs et nerveux, et des muscles chez les rats et les lapins. Les sinus nasaux et les poumons ne sont pas atteints.

Par inhalation à la concentration de 15 mg/m^3 de poussières métalliques de nickel de dimensions inférieures ou égales à 4 μ pendant une période maximale de 21 mois, tous les cobayes et presque la totalité des rats ont des tumeurs soit malignes, soit bénignes au niveau du poumon. Les souris, par contre, ne présentent aucune modification histologique au niveau pulmonaire.

Chez le cobaye, le poumon présente des modifications très nettes sous forme de proliférations épithéliales dans les muqueuses bronchiques et étendues dans plusieurs zones aux alvéoles adjacentes : quelques-unes de ces lésions ressemblent aux carcinomes des bronchioles et aux adénosarcomes (en miniature) des alvéoles avec un carcinome alvéolaire anaplastique bien défini chez un animal.

Chez le rat, on observe les mêmes formations adénoïdes au niveau des alvéoles et en plus des sarcomes des nodules lymphatiques abdominaux et médiastinaux.

Enfin l'action du nickel sur le système cardiovasculaire, sur l'appareil respiratoire, sur l'appareil digestif et les glandes annexes, sur le système nerveux, sur le sang et sur les glandes à sécrétion interne est bien décrite par ROYER [8].

Chez l'homme, aucun empoisonnement systématique n'est signalé au cours de l'utilisation du nickel soit en thérapeutique, soit dans le domaine industriel.

En thérapeutique, le nickel est utilisé vers la fin du 19ème siècle contre les anémies et comme sédatif du système nerveux contre les maux de tête, les névralgies et les insomnies, soit sous forme de sulfate, soit sous forme de bromure.

Aux doses supérieures aux doses thérapeutiques usuelles, on observe quelques étourdissements, quelques nausées et vomissements.

Dans le domaine industriel, les dérivés métalliques du nickel provoquent très souvent des dermatoses ; mais le composé responsable de nombreuses intoxications est le nickel carbonyle. La symptomatologie et les lésions provoquées par ce dérivé sont bien décrites dans les livres spécialisés de toxicologie industrielle (voir BROWNING, 1969 [24]).

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J. Occup. Med.
9(3) 127-36 (1967)

Nickel: A Review of Its Occupational Health Aspects

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ONTARIO has large deposits of nickel ore in the Sudbury area and from these produces about 70% of the free world's consumption of nickel. Because of this, the occupational health aspects involved in mining and refining nickel ores and in the industrial use of nickel alloys and nickel compounds assumes special importance for us in Ontario.

Nickel is a grayish-white metallic element—hard, tough, and markedly resistant to oxidation and corrosion. Meteoric iron contains 5–15% nickel, and this alloy was used for tools in prehistoric times. Nickel was first isolated in impure form in 1751 by A. F. Cronstedt in Germany from an ore containing niccolite (NiAs). An ore of this type had earlier caused trouble with the copper and silver mines in Saxony because it yielded an unusual brittle product. It was referred to as "kupfernickel" after "Old Nick" and his mischievous gnomes, and Cronstedt applied the name "nickel" to his new element.[1]

Although nickel is a fairly common minor constituent of igneous rock (it constitutes about 0.016% of the earth's crust), there are few deposits that qualify with respect to concentration, size, and accessibility for commercial interest. The most important sources of the metal are the mixed sulfide ores containing pentlandite ($NiFeS_2$) as the principal nickel mineral; nickeliferous pyrrotite (Fe_7S_8); and the copper-bearing mineral chalcopyrite ($CuFeS_2$). Other metals, present in small amounts, include cobalt, selenium, tellurium, silver, gold, and the metals of the platinum group. These ores are mined on a large scale at Sudbury, Ontario, and Thompson, Manitoba. The ore is of variable grade and may average 2–3% copper-nickel.

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Processing and refining of the ore is costly and complex and has undergone many modifications. The bulk of the ore is dry-crushed, sized, and wet-ground in rod-and-ball mills. It is passed through a series of classifiers, flotation cells, thickening tanks, filters, and magnetic separators. Three sulfide concentrates are produced: copper, nickel concentrate containing some copper, and iron. The copper concentrate is sent to the copper smelting furnace, the nickel concentrate to the smelter roasting furnaces, and the iron concentrate to the iron-ore recovery process.

The nickel concentrate is roasted in multi-hearth roasters to eliminate about one-half of the sulfur and to oxidize the iron associated with this sulfur. The resulting hot calcine is smelted in pulverized coal- and natural gas-fired furnaces. The furnace matte is blown with air in Pierce-Smith converters to remove iron and its associated sulfur. The sulfur is oxidized to sulfur dioxide, forming part of the fuel in the process. The iron is removed by the formation of a slag with added silica. Molten matte from the converters, consisting essentially of copper and nickel sulfides, is poured into shallow molds and allowed to cool slowly. The matte is then crushed. The crushed matte is wet-ground and magnetically separated to recover a concentrate containing the bulk of the precious metals. Subsequently, the fine, nonmagnetic fraction is floated to separate copper sulfide from nickel sulfide. Most of the nickel sulfide from this separation was formerly sintered but is now fluid-bed roasted to produce nickel oxide.

Details of nickel refining operations (Mond process) carried out in Great Britain have been published by Morgan.[2] In Ontario, fluid-bed nickel oxide is reduction smelted in reverberatory furnaces and cast into anodes. The anodes are electrolytically refined. The pure cathode metal is sheared, packed, and shipped, or melted to produce nickel ingot or shot. The electrolytic sludge (or anode residue) is further processed for recovery of precious metals.

Estimates of the consumption of nickel during 1965 in the various principal fields of application are shown below.

Field	1965 Consumption Millions of lb.	%
Stainless steel (2–26% Ni)	247	33.8
Nickel plating	111	15.2
High nickel alloys (e.g., Monel 65–70% Ni)	101	13.8
Construction alloy steels (2–10% Ni)	91	12.5
Iron and steel castings (e.g., ductile iron 1–5% Ni)	80	10.9
Copper and brass products (e.g., nickel silvers 10–30% Ni)	31	4.3
All others	69	9.5
TOTAL	730	100.0

Nickel and its alloys are used in coinage (25–100%). Many other nickel alloys are commercially available. These include heat-resistant alloys (75% Ni and 14% Cr); electrical-resistance alloys (80–85%); and permanent-magnet alloys (14–32%).

Nickel compounds of commercial importance include: nickel oxide (nickelous oxide, NiO), used in the preparation of nickel alloys, ground-coat enamels, and in coloring ceramics and glass; nickelous hydroxide ($Ni(OH)_2$), a light-green powder used to prepare nickel catalysts and in nickel-iron-storage batteries; nickel sulfate ($NiSO_4 \cdot 6H_2O$), the most familiar nickel salt used in electroplating baths; nickel chloride ($NiCl_2 \cdot 6H_2O$), and nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$), used in electroplating and in the preparation of nickel catalysts.

Nickel carbonyl ($Ni(CO)_4$) is prepared by treating finely divided metallic nickel with carbon monoxide. Sulfur acts as a catalyst for this reaction. The Mond refining process makes use of this carbonyl to produce highly refined nickel. Nickel carbonyl is very toxic.

Nickel has important uses as a catalyst. Various forms and compounds are used, e.g., nickelous hydroxide, nickel nitrate, and nickel formate, all of which yield finely divided nickel in an active form. Nickel catalysts are most frequently used in hydrogenation of fats and oils. The significance of residues of metallic nickel in food resulting from its use as a hydrogenation catalyst has been raised.

Toxicity of Nickel and Its Compounds

The toxicity of nickel and its compounds has been reviewed by Stokinger.[3]

Nickel salts are highly toxic when administered intravenously or subcutaneously. Colloidal nickel and nickel chloride are lethal to dogs in single intravenous doses of 10–20 mg./kg. Soluble nickel salts are lethal in subcutaneous doses of 7–8 mg./kg. to rabbits and 9–16 mg./kg. to cats.

Ingested nickel metal is relatively nontoxic. There is a high oral tolerance in dogs; oral doses of 1–3 gm./kg. are without effect. Doses of 4–12 mg./kg. daily for 200 days in dogs and cats produced no ill effects. There is no evidence that nickel absorbed from food or from food cooked in nickel or nickel alloy containers is of any health significance even though a few milligrams may be ingested daily.[4]

In metabolic studies in dogs it was found that 90% of ingested nickel was excreted in the feces and 10% in the urine. Blood and urine values for nickel have been published.[5] The normal values for nickel in blood and urine have been reported as 3 μg./100 ml. and 7.6–11 μg./L. respectively.[6,7] Those exposed to nickel may show blood values ranging from 16 to 225 μg./100 ml. Normally no nickel is present in the liver. The lungs contain from 0.4 to 22 μg./100 gm. of wet tissue.

Nickel carbonyl ($Ni(CO)_4$) is extremely toxic. It is a mobile, colorless liquid with a high vapor pressure. It boils at 43° C. and begins to decompose at 60° C., producing finely divided nickel and carbon monoxide. It is used in the Mond process for refining nickel. It may also be encountered in other operations in which nickel and carbon monoxide are present and capable of combining, e.g., inert gas-shielded arc welding with carbon dioxide gas on Monel metal, and flushing out nickel vessels with "inert gas" containing carbon monoxide, according to Eckardt.[8] The LC_{50} for a 30-min. exposure in rats is 240 mg./cu.m. (about 35 ppm). Symptoms of acute exposure include: giddiness, headache, dyspnea, and vomiting. Commonly, victims feel better when they are removed to fresh air. However, 12–36 hr. later dyspnea returns, and symptoms become much more severe, with cyanosis, leukocytosis, fever, cough with blood-tinged sputum, delirium, and progressive respiratory difficulty. Death may occur in 4–11 days. Postmortem findings in fatal cases are similar to those produced by inhalation of phosgene, with evidence of an acute chemical pneumonitis. Calcium disodium EDTA has been tried without success in nickel carbonyl poisoning. BAL provides only partial success. The therapeutic agent of choice according to Sunderman and Sunderman[9] is Dithiocarb (sodium diethyldithiocarbamate trihydrate).

Kincaid *et al.*[6] suggested 0.04 ppm as the maximum allowable concentration for nickel carbonyl to prevent acute effects, but because of its implication with lung cancer, a threshold limit value of 0.001 ppm has been established by the Threshold Limit Values Committee of the American Conference of Governmental Hygienists.[10] Table 1 shows the guidelines proposed by Kincaid and his associates.[6]

Nickel Dermatitis

Dermatitis from contact with nickel-plated articles, jewelry, garter buckles, and coins has been known for some time. Numerous cases of dermatitis have been reported among workers engaged in electroplating with nickel and in refining nickel. The typical "nickel itch" usually begins with a burning and itching of the exposed skin on the hand. Erythema appears and later a nodular eruption in the web of the fingers and in the forearms. These nodules may go on to form pustules. Recovery is common in 1 week, but the rash may persist for weeks in some instances. There are two components with nickel dermatitis—a simple dermatitis limited to the area of contact followed by a chronic eczematous reaction that becomes widespread.

Bulmer and MacKenzie[11] studied "nickel rash" in Ontario nickel refinery workers, and they found that heat and moisture were the most important contributing factors. Cases were more numerous during hot weather when sweating was increased or when work was done under hot, moist conditions. These authors administered calcium chloride by mouth in the hope of affecting the pH of the sweat, and they felt that this was useful in many cases.

Fisher and Shapiro[12] noted that nickel was a common cause of allergic eczematous contact dermatitis and that more cases of skin trouble became apparent with the wider use of nickel. They re-examined 40 patients with nickel sensitivity and found that 36 of them were still sensitive to it on patch-testing 2–17 years after the original diagnosis. They used 10% nickel sulfate but recommended that a 5% concentration was preferable for patch-testing. Nickel should be regarded as a relatively potent skin sensitizer. It is quite specific; Fisher and Shapiro found no cross sensitization in their patients on patch-testing with 2% cobaltous sulfate, 0.5% potassium dichromate, or 5% copper sulfate.

TABLE 1. THRESHOLD LIMIT VALUES FOR NICKEL CARBONYL*

	Concentration (ppm)		
	In plant		
	Single air sample	*Daily average concentration*	*Outside plant*
Target value	0.04	0.001	0.003
Discontinue operations or respiratory protection required	0.2–2.0	0.001–0.005	—
Shut down operations	2.0	0.005	0.001

*From Kincaid *et al.*[6]

Respiratory Disorders from Nickel

Certain respiratory effects have been reported among workers exposed to nickel fumes and dust but such reports are clouded by concomitant exposure to other substances. There have been reports of trouble when nickel was used in such operations as polishing, electroplating, nickel-cadmium battery manufacture, and welding. Cases of acute pneumonitis as a result of exposure to nickel fume have been described.[13] In these reports it is not possible to rule out the effects of exposure to such agents as ozone, nickel carbonyl, or the products of decomposition of trichloroethylene.

In general, there is no real evidence that nickel fume or dust produces any particular inflammatory disease or fibrosis of the lung in workers on either acute or chronic exposure.

Respiratory Cancer in Nickel Refinery Workers

The production of nickel by the gaseous carbonyl process began at a refinery in Wales in 1902. This plant was the first of its kind in the world. In 1932 Bridge[14] reported 10 cases of cancer of the interior of the nose that had occurred at this refinery after 1923. He commented guardedly that the question of industrial responsibility must remain open "but the very high incidence of cancer in a little-recorded site cannot be regarded otherwise than as suggestive." Amor, at that time the medical officer of the nickel refinery, undertook a complete medical survey. In 1938 he reported[15] that 21 workers engaged in extracting and refining copper and nickel had developed cancer of the respiratory tract. Ten of these cancers were in the ethmoid region and 9 involved the middle turbinate bone. Histologically, 6 of the nasal cancers were undifferentiated, 3 were squamous-cell carcinoma, and 1 was a papillary (columnar-cell) carcinoma. Amor believed that arsenic was responsible. The smelted ore contained about 2% arsenic, and the sulfuric acid used in extraction prior to 1924 was contaminated with arsenic. Bradford Hill,[16] after a statistical investigation in 1939, concluded that there was a large excess of deaths from cancer—almost entirely among process workers—at two main sites, the lung and the nose.

In Great Britain, however, cancer of the lung and nose was not made a prescribed disease (i.e., compensable) until 1949.[17] This occurred after Barrett,[18] in his annual report for 1948, again drew attention to the increase in these cancers among nickel refinery workers. For the period 1923–1948, 47 cases of nasal cancer and 72 of lung cancer were reported from the Welsh nickel refinery. The average number of years worked before nasal and lung cancer occurred was 23 and 25 years respectively. The official inclusion of nickel as an industrial carcinogen in Great Britain was clearly expressed in the list of prescribed diseases. The disease was described as: (1) cancer of the mucous membrane of the nose or associated air sinuses, and (2) primary cancer of a bronchus or of a lung, and the nature of the carcinogen was given as "nickel produced by decomposition of a gaseous nickel compound."

Subsequent experience at the Welsh refinery, brought up to date in a series of articles in 1958 by Morgan,[2] Doll,[17] and Williams,[19] confirmed the earlier observations. Doll noted that for the years 1948–1956 "the risk of nickel workers dying from lung cancer was approximately five times 'normal'" and for the same period their risk of dying from nasal cancer was 150 times normal. Doll noted that there was no excess mortality apparent in workers less than 50 years of age, and he felt that it was reasonable to believe that the hazard had been largely or completely removed after 1924. No deaths from nasal cancer have been recorded from workers commencing employment after 1924, and this confirmed Doll's statement.

Morgan[2] reported that there were 131 cases of lung cancer and 61 of nasal cancer among nickel refiners for the period 1923 to 1957. A good description of the nickel carbonyl process is given in his article. Workers showing an increased incidence of respiratory cancer were those involved in dusty operations rather than those exposed to nickel as a gaseous compound. Cancer of the nose was considered far more significant than cancer of the lung as an index of action of the carcinogenic material. Morgan postulated that the carcinogenic material was present in heated calcined dusts and he stressed the arsenic content. Arsenic-free sulfuric acid was used after 1924, and he attached importance to this. Other precautions were also taken at this time, however, and were probably of more significance. These included modernization of equipment, especially the calciners; improved ventilation and dust control; and the use of respirators in dusty areas.

Williams[19] examined histologically and chemically the lungs of 5 men who had worked at the Welsh refinery. Four of the 5 had pulmonary carcinoma (3 squamous-cell and 1 alveolar-cell); the fifth worker did not. There was evidence of long-standing inflammation in 4 of the lungs. All showed varying degrees of nonspecific diffuse interstitial fibrosis; this was probably of infective and not industrial origin. Nickel and copper were present in excess in the lung tissues of the 2 cases studied, but no arsenic could be detected. His findings on the chemical analysis may be of interest (Table 2). Williams found no particular diagnostic or precancerous epithelial changes. Although the evidence was inclusive, exposure to nickel, to-

TABLE 2. CHEMICAL ANALYSIS OF LUNG TISSUE FROM 2 NICKEL REFINERY WORKERS WITH LUNG CANCER*

	Lung ash (%)	*Nickel in dried lung (ppm)*	*Arsenic in dried lung (ppm)*	*Copper in dried lung (ppm)*
Controls†				
Coal miners	14.95	< 5	< 0.2	31
Nonminers	14.1	< 5	< 0.2	29
Case 4	12	90	< 0.2	460
Case 5	20	120	< 0.2	2810

*From Williams.[19]

†Nickel controls were 25 coal miners and 25 nonminers; arsenic and copper controls were 10 coal miners and 10 nonminers.

gether with chronic inflammation of the lung, were considered by him as the responsible carcinogenic factors.

Løken,[20] in 1950, noted an increase in respiratory cancer among furnace workers in a Norwegian nickel refinery in which electrolytic refining of nickel was carried out. He reported 3 cases of lung cancer. The 3 affected workers had had pneumonectomies, and bronchogenic carcinoma was confirmed in all. In 1, other complicating findings were noted. These included localized fibrosis, the presence of asbestos bodies, and changes typical of Boeck's sarcoid. This man had worked for 10 years at the roasting furnaces and then had been away from nickel exposure for 8 years. Despite this long period without exposure, the amount of nickel in his lung was found to be between 1000 ppm and 2800 ppm. This result is extremely high and is open to question. The 2 other men were furnace workers for 22 years. Later Løken reported 2 additional workers from the same nickel refinery with lung cancer between 1950 and 1955 in a personal communication to Goldblatt and Goldblatt.[21]

Rockstroh[22] noted an increase in respiratory cancers in German workers exposed to dust and fumes from ores containing nickel, bismuth, cobalt, and large amounts of arsenic in a nickel refinery. The author suggested that, on the basis of primary irritation, dusts and vapors might be responsible for bronchitis and bronchial carcinoma developed later as a result of the influence of inhaled arsenic, nickel, and cobalt.

Because of the reported increase in respiratory cancer among process workers in the nickel refinery in Great Britain, Sutherland[23] carried out a detailed study of mortality in workers employed in an Ontario nickel refinery. Operations began in the refinery in 1918, and the present work force numbers about 2000 workers. Sutherland's study covered the period 1930 to 1957. Workers were divided into 8 exposure groups as follows:

1. Furnace workers
2. Workers exposed to other dust, e.g., maintenance staff
3. Electrolytic workers
4. Nonexposed workers, e.g., storeroom workers
5. Office workers
6. Mixed exposure, e.g., workers with less than 3 years in Groups 1 and 2 plus other work
7. Mixed exposure, e.g., workers with 3 years or more with furnace exposure (Group 1) plus other work
8. Mixed exposure, e.g., workers with 3 years or more with other dust exposure (Group 2) plus other work

The workers were also divided into groups based on the duration of exposure as follows: (1) 25 years or more, (2) 20–24 years, (3) 15–19 years, (4) 10–14 years, and (5) 5–9 years.

Only workers with 5 or more years of exposure were included in the study. In all, a total of 2355 workers were included, providing 32,342 employee years at risk and 10,295 years not at risk (i.e., retired). In these workers there were 245 deaths from all causes between 1930–1957.

Age-specific male death rates in Ontario for specified causes were used to calculate the expected numbers of death in the study group. The results of this investigation are shown on Table 3. In general, they confirm the findings reported from Great Britain where increase in mortality from sinus and lung cancer was found in dustry exposures.

Sinus Cancer

All deaths from sinus cancer occurred since 1946 in workers employed before 1934. Furnace workers showed the greatest risk. Those with 3 years or more exposure had a death rate from sinus cancer 200 times greater than "normal" rate (which is less than 1:100,000 in the general population). The risk of mortality from sinus cancer increased with the duration of furnace exposure—at least up to the levels of 20 years or more of exposure. The data were strongly suggestive that exposure of even 1 year or less in cupola furnace operations was associated with increased risk of sinus cancer. There was also good evidence to indicate an increased risk after 3 years or more in sinter plant workers. There was no definite evidence that employment solely about the calcining furnaces contributed to sinus cancer. Workers at the anode

TABLE 3. MORTALITY IN ONTARIO NICKEL REFINERY WORKERS 1930–1957

Cause of death	*No. deaths* Observed	Expected
Sinus cancer	7	0.19
Pulmonary cancer	19	8.45
Cancer, all sites	54	43.19
Vascular lesions of CNS	14	20.72
Respiratory diseases	13	16.21
Gastrointestinal diseases	9	16.07
All causes	245	308

furnace, in the electrolytic plant, in nondust exposure, and in offices showed no increased risk.

Lung Cancer

All deaths from lung cancer occurred after 1946. There is good evidence of an increased risk of mortality for men employed in furnace operations other than the anode furnace for 25 years or more. There is suggestive evidence of an increased risk after 3 years or more of furnace exposure. Employment of 6 months or more at the cupola furnace and 3 years or more at the sinter plant produced an increased risk of death from lung cancer There was no real evidence of any increase in electrolytic workers, nondust workers, and office workers. The risk of death from lung cancer in nickel process workers is about 2.25 times greater than "normal" (3.6 times for furnace workers with 3 years or more of exposure).

An additional 9 sinus cancer and 16 lung cancer deaths occurred in the period 1958–1965 among men employed prior to 1940. Table 4 shows the number of deaths from sinus and lung cancer by exposure group for the period 1930–1965. As a result of the investigation by Sutherland in 1957–1958 major changes were made in the process to eliminate those exposures that were shown to offer increased risk of respiratory cancer. Cupola furnace operations had been eliminated previously. The sinter plant operation was terminated in 1962. Calcining operations were drastically curtailed.

In 1965 Tatarskaya[24] reported 2 cases of cancer of the nose and paranasal sinuses in workers engaged in the electrolytic purification of nickel in the USSR. During their work they were exposed to dust containing nickel, cobalt, copper, iron, and other substances. Little information is given in the abstract with respect to the nature of the exposure—in particular, whether furr exposures were involved.

Experimental Data

The specific carcinogen responsible for increase in sinus and lung cancer in nickel finery workers is unknown and it is the sub of continuing research. Finely divided meta nickel was shown to be carcinogenic to when injected into the femoral marrow cav the pleural cavity, the nasal sinus, intram cularly, or subcutaneously.[25,26] This kind of perimental carcinogenesis is open to much q tion ever since Schinz and Uelinger[27] showed in 1941 that injection of arsenic, cl mium, and cobalt into the femur of rabbits p duced metatasizing sarcomas and an adeno cinoma. Heath[28,29] reported that metallic balt injected intramuscularly produced sarc in rats. Nothdurft[30,31] induced sarcomas in by the subcutaneous implantation of glass, discs of plastic and various precious met Nothdurft called these "foreign-body sarcom and considered that they were invariably fil sarcomas developing after a long latent per from cells of the connective tissue walling disc. This does not, however, explain the occ rence of tumors remote from the site of in tion.[25]

The factors affecting carcinogenic act were listed by Hueper and Conway[32] as p tein-binding and chelation, electronegativ and ionic radius, physical form, solubility, tribution in the periodic table, and poly character. Payne[33] noted that the highly solu nickel compounds were rapidly excreted were not carcinogenic; the insoluble nickel co pounds, e.g., nickel sulfide, when injected mained at the site and produced sarcoma i high proportion of the rats treated.

Metallurgical dust obtained from the flue

TABLE 4. MORTALITY IN ONTARIO NICKEL REFINERY WORKERS BY EXPOSURE GROUPS 1930–1965

	Sinus cancer deaths		Pulmonary cancer deaths	
Exposure group	*Observed*	*Expected*	*Observed*	*Expected*
1 Furnace workers	5	0.023	8	1.88
2 Other dust	—	0.029	4	1.89
3 Electrolytic workers	—	0.014	1	1.26
4 Nondust	—	0.011	—	1.07
5 Office	—	0.006	2	0.41
6 Mixed $<$ 3 yr. in 1 & 2 + other work	2	0.035	6	2.57
7 Mixed $\geqq$ 3 yr. in 1 + other work	8	0.026	15	2.15
8 Mixed $\geqq$ 3 yr. in 2 + other work	1	0.022	1	1.47
TOTAL	16	0.166	37	12.711

an Ontario nickel refinery was shown to be a locally acting carcinogen in both rats and mice.[34] Sarcomas were produced in 2 strains of rats at about 45% of the sites of a single intramuscular injection, with most of the tumors being of striated-muscle origin. The average latent period was 6 months, and metastases occurred frequently. Mice were much less responsive than rats. Gilman and Ruckerbauer[34] also found that a single intramuscular injection of cobalt oxide gave a 50% incidence of local rhabdomyosarcomas in rats but was completely negative in mice. An analysis of the nickel refinery dust that they used is shown in Table 5.

Single intramuscular injection of nickel sulfide (Ni_3S_2), nickel oxide (NiO), cobalt sulfide (CoS), and cobalt oxide (CoO) in rats were shown to be carcinogenic by Gilman.[35] Sulfides induced a significantly higher tumor incidence than the oxides. The presence of sulfide seemed to enhance the carcinogenic activity—perhaps by alteration in solubility and/or binding power of the metal compounds. Nickel sulfate ($NiSO_4 \cdot 6H_2O$), iron oxide (Fe_2O_3), and copper oxide (CuO) were not carcinogenic in rats on intramuscular injection. Gilman noted that direct extrapolation between the induction of rhabdomyosarcomas in rats and the occurrence of sinus and lung cancer in refinery workers is not justifiable.

The inhalation of finely powdered metallic nickel at a concentration of 15 mg./cu.m. for 6 hr. daily 5 days a week over a period of 21 months was associated with the production of benign and malignant pulmonary neoplasms in guinea pigs and pulmonary adenomatoid proliferations in rats.[36] Sunderman and his associate exposed rats to nickel carbonyl. After 1 year of exposure the rats showed extensive pulmonary changes, including "a remarkable degree of squamous metaplasia of the bronchial epithelium."[37] Later they found that pulmonary cancers were induced in rats subjected to a heavy single exposure to nickel carbonyl as well as to repeated sublethal exposures over a period of 1 year.[38] Their results are shown in Table 6. The numbers involved are small, but the authors concluded that "the inhalation of nickel carbonyl can cause pulmonary cancer in rats."

Hueper and Payne[39] administered finely powdered nickel intratracheally in rats and produced cancer in one-third of the animals treated. Intrapulmonary deposition of fine nickel powder produced sarcoma in only 1 of 34 animals exposed. In another experiment rats and hamsters inhaled finely divided nickel powder and sulfur dioxide together. No tumors were produced by this combination, and these workers concluded that a purely irritative gas inhaled with a carcinogen did not induce respiratory cancer.

Extensive inhalation experiments are being conducted at the School of Hygiene in Toronto by Fisher.[40] Nickel refinery dust similar to that used by Gilman and Ruckerbauer[34] was used. No real evidence of the induction of pulmonary tumors after chronic inhalation was found in the animals used, including rats, mice, and pigeons. Pigeons were used to study the effects of inhalation on the mucosa of air sinuses. Fisher also used other dusts as follows: a synthetic dust (containing 34.1% $NiSO_4 \cdot 6H_2O$, 68.7% Ni_3S_2 and 7.2% NiO), iron sulfide (FeS), cobalt sulfide (CoS), nickel sulfide (Ni_3S_2), and the

TABLE 5. ANALYSIS OF FLUE DUST FROM ONTARIO NICKEL REFINERY*

Substance	*Per cent*
Cupric oxide (CuO)	3.4
Nickel sulfate ($NiSO_4 . 6H_2O$)	20.0
Nickel sulfide (Ni_3S_2)	59.0
Nickel oxide (NiO)	6.3
Cobalt oxide (CoO)	1.0
Ferric oxide (Fe_2O_3)	1.8
Silicon dioxide (SiO_2)	1.2
Moisture	7.3
Other†	2.0

*From Gilman and Ruckerbauer.[34]

†Pb, As, Al_2O_3, CaO, MgO, and Na in amounts from >0.01 to $<0.1\%$; Bi, Ag, Te, Cr, Au, and B in amounts of $<0.01\%$.

TABLE 6. PULMONARY CANCER IN RATS EXPOSED TO NICKEL CARBONYL*

Rat group	*Nickel carbonyl concentration (mg./cu.m.)*	*Duration exposure*	*No. at start*	*No. alive after 24 mo.*	*No. with pulm. ca.†*
C	0.0	3 × wk. for 1 yr.	41	3	0
X	30	3 × wk. for 1 yr.	64	5	1
Z	60	3 × wk. for 1 yr.	32	1	1
EP	250	Single exposure	80	3	2

*From Sunderman *et al.*[38]

†Number of rats with pulmonary cancer of those living after 24 months.

refinery flue dust to which 1% As_2O_3 was added. Exposure concentrations varied; most were in the range of 5 to 15 mg./cu.m. The synthetic dust containing nickel sulfate was found to be the most toxic. Cobalt sulfide was not associated with the occurrence of lung tumor or squamous metaplasia; the other dusts used were associated with the appearance of these in some animals. Eleven of 348 exposed rats developed lung tumors, as did 3 of 85 controls. Sixteen of the exposed rats showed squamous metaplasia of the bronchial epithelium; this was also observed in 1 rat in the control group. Nothing significant was noted in the histological examination of the sinuses of the pigeons used. In general, the chronic inhalation of nickel refinery dust, a synthetic dust, iron sulfide, and nickel sulfide was associated with the occurrence of lung tumors and squamous metaplasia in rats, but the significance of this is difficult to assess because of the occurrence of similar changes in control animals.

Belobragina and Saknyn[41] exposed rats by intratracheal installation and inhalation to furnace dusts from a nickel smelting plant in the USSR. The sinter dust, which contained about 65% nickel sulfide and nickel oxide, produced connective-tissue nodules; electric furnace dust containing about 95% nickel oxide produced a diffuse pulmonary sclerosis. No tumors were produced.

In connection with the relationship between pulmonary cancer and exposure in a nickel refinery, the finding by Wase and his associates[42] is of interest. Using radioactive nickel, they found that it had a high value for the complex formation constant with lung protein in the mouse. The reasons or even the significance for the affinity of nickel for lung protein are not understood.

Discussion

Doll,[43] in a review of occupational lung cancer, classified exposure risks as established, suspected, or possible. Table 7 is adapted from his article. He noted that the only lung cancer prescribed as a definite occupational disease in Great Britain is that which occurs in nickel workers exposed to nickel powder formed "by decomposition of a gaseous nickel compound." It was suggested by Goldblatt and Goldblatt[21] that tumor production was associated with nickel in some especially active or finely divided form characteristic of its liberation from nickel carbonyl. They went on to say, "This is not an indictment of nickel in general as a carcinogen for there is no evidence that it is active in this way in its usual uses and applications." Finely divided nickel is known to have special properties that the metal does not have.

Hueper and Conway[32] noted that the smelting and refining of nickel presented a recognized risk in producing respiratory cancer in workers engaged in these operations. They noted further, however, that there is a suspected risk in nickel ore mining; shipping, loading, and grinding of nickel; nickel carbonyl production; production of Monel metal; production of stainless steels; nickel alloy production; nickel plating; hydrogenation of vegetable oils; coin makers—in fact any operation in which nickel is used. These authors also listed the nonoccupational use of nickel-containing kitchen utensils and tableware as suspected risks. I find absolutely no evidence or basis for this concern. There is no evidence of any increase in respira-

TABLE 7. OCCUPATIONAL RESPIRATORY CANCER*

Risk category	*Exposure group*	*Degree of risk*	*Suspected carcinogen*
Established	Schneeberg miners	Lung 10 × normal	Radioactivity; radon
	Nickel refinery workers	Lung 5 × normal Nose 150 × normal	Calcined dust containing nickel
	Chromium refiners	Lung 3½–30 × normal	Acid-soluble, water-insoluble trivalent chromium compound
	Asbestos workers	Lung	Asbestos dust
	Gas production workers	Lung 15 × normal	Coal tar
Suspected	Miners and metal refiners	Lung 10 × normal	Arsenic
	Iron and steel workers	Lung	Iron dust & fumes
Possible	Chemical plant operators	Nose & lung	Isopropyl alcohol intermediate
		Lung	Beryllium
	Copper mining and copper smelting	Lung up to 10 × normal	Copper dust?

*Adapted from Doll, R.[43]

tory cancer among Ontario nickel miners. Similarly, it may be significant to note that no increase in respiratory cancer has become apparent in the huge Sudbury smelter complexes that have been processing the nickel ore to nickel matte since the turn of the century. Further studies on this exposure group are continuing. The increase in respiratory cancer noted in the reported literature has been only in nickel refiners exposed to furnace dusts and fumes containing nickel.

There is perhaps some analogy between the increase in respiratory cancers noted for chromium refinery workers and that for nickel refinery workers. In both cases, there is no evidence that the metal or its compounds used in commerce have been associated with any increased risk. Furthermore, there is no evidence that residues of nickel associated with its use as a hydrogenation catalyst or in cooking utensils have any health significance. They certainly do not present any carcinogenic risk.

The experimental evidence reviewed earlier demonstrates that insoluble nickel compounds are carcinogenic when administered by injection to experimental animals. Inhalation of nickel and its compounds in animals has not yielded significant results. The epidemiological evidence from studies of workers engaged in nickel refining points to an association between the inhalation of freshly heated insoluble dust and/or fume and increased risk of respiratory-tract cancer.

In nickel refining most of the data show an association between furnace operations and increased risk. Furthermore, duration of exposure is significant and this may be evidence of a dose-response relationship.

In this regard the air concentrations of nickel-containing dust and fume in nickel refining operations are important. The concentration of nickel in air that is believed to present an increased risk of respiratory-tract cancer in nickel refinery workers is not known. Much depends on the nature of the actual carcinogen itself. A threshold-limit value of 1 mg./cu.m. for nickel and insoluble compounds of nickel has been published.[10] In my opinion, this threshold-limit value is sufficiently low to prevent any significant respiratory cancer risk. However, further studies are indicated. Improvements in equipment and dust control measures in Great Britain resulted in elimination of the risk of nasal cancer in workers entering employment after 1924. In Ontario, respiratory cancer has been reported in workers entering employment from 1918 to 1944. During this period conditions were very dusty, particularly during the war years. As previously indicated, process changes designed to eliminate exposures associated with increased risk were instituted.

Acknowledgment

I should like to express my gratitude to Dr. R. B. Sutherland, Director of the Environmental Health Branch, Ontario Department of Health, for his very helpful advice and criticism during the writing of this review.

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STATISTICS ON AGING

Older people have been increasing in proportion to the rest of the population for over 100 years. Since 1950, the percentage of age 65 and over has risen from 8.1 to 9.3%. From now on the increase will be much slower because of the increased birth rate. Women outnumber men at all age brackets, but the disproportion is greater in old age. There are now 129 women for every 100 men at ages 65 and up. Twenty years from now the odds may be as high as 143 to 100.

*J Indiana Med Ass 59:*256, Mar. 1966.

J. Nutr
100:1447-53 (1970)

Nickel Toxicity in the Young Bovine [1]

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ABSTRACT Twenty-three male dairy calves were fed a) 0, b) 62.5, c) 250, or d) 1000 ppm elemental nickel (Ni) as $NiCO_3$ in the total diet from 13 to 21 weeks of age. Three animals on each treatment were subjected to digestion and balance trials and killed for tissue histological comparisons, and a like number (two for 62.5 ppm) were fed the basal ration in a 6-week posttreatment recovery period. Feed intake and growth rate were slightly retarded by 250 ppm Ni. Even though the calves fed 1000 ppm Ni had greatly reduced feed intake and lost weight during the 8-week treatment period, they were not emaciated and appeared to be younger than the others. During the posttreatment recovery period, growth rate of those which had been given 1000 ppm Ni was at least equal to that of the others. Digestibility coefficients were not affected by the Ni treatments but nitrogen retention was significantly lowered by 1000 ppm Ni and was associated with reduced feed intake. On a molar percentage basis, propionate was increased and butyrate decreased in rumen fluid from animals on the higher levels of nickel supplementation. Relative to body weight, the fresh weight of lung, heart, spleen, liver, gall bladder, kidney, brain, and testis was unaffected by treatments. Nickel did not affect ruminal, abomasal, duodenal, liver and testicular tissues histologically. Kidneys were nephritic, and the degree of severity increased with nickel level. J. Nutr. *100:* 1447–1454, 1970.

In spite of repeated in vitro demonstrations of enzyme activation properties an essential biological role for nickel has not been established in plants or animals (1–5). Nickel is present in ribonucleic acids from various biological sources (6, 7) and may have carcinogenic properties (8, 9). Nickel toxicity occurs to varying degrees in both plants and animals. Nickel in plant tissues may be toxic at 40 to 60 ppm (10). Normally insoluble nickel compounds become soluble in soil of low pH, which causes nickel to accumulate in plants (11), but adding lime to nickel-treated soil counteracts the toxic effect on plant growth (12).

Nickel, as acetate or sulfate, at 700 ppm or above significantly depressed chick growth to 4 weeks of age and decreased metabolizable energy and nitrogen retention (13). With female mice, 1100 and 1600 ppm Ni as acetate in the diet reduced growth (14).

Intraperitoneally injected ^{63}Ni was detected in numerous mouse tissues, but for lung and brain it apparently was rapidly excreted (15). Intravenous injections of ^{63}Ni into rats resulted in wide tissue distribution but only the kidney contained significant amounts of ^{63}Ni 72 hours later (16).

A literature survey has revealed only one reference to nickel supplementation of ruminant rations (17). Archibald fed 145 mg of elemental nickel daily as nickelous chloride to lactating dairy cows without obvious harmful effects (17). This level of nickel was below that at which toxicity symptoms were later shown in non-

Received for publication July 10, 1970.

[1] Technical contribution no. 871, South Carolina Agricultural Experiment Station; published by permission of the Director. University of Georgia, College of Agricultural Experiment Stations, Journal paper no. 828, College Station, Athens.
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ruminants (13, 14, 18, 19). Thus, this study was initiated to evaluate the effects of high nickel levels on growth, feed utilization and tissue aberrations.

EXPERIMENTAL PROCEDURE

Twenty Holstein and three Brown Swiss male calves born during a 5-month period, were sequentially assigned, prepartum, to one of four treatment groups. The calves were fed a limited amount of milk to 7 to 8 weeks of age and grain and hay from 4 to 12 weeks of age at which time they were drenched for parasites with thiobendazole. At the beginning of week 13 the calves were fed the experimental basal diet during a 1-week preliminary period. At the end of the preliminary period, each animal received one of four dietary treatments consisting of the basal diet plus elemental nickel supplementation of 0, 62.5, 250 or 1000 ppm as nickelous carbonate ($NiCO_3$) for 8 weeks. The animals were fed ad libitum twice daily with refusals weighed daily. The basal diet, on a percentage basis, consisted of the following: ground yellow corn, 42.1; oats, 18.9; soybean oil meal, 13.2; alfalfa leaf meal, 2.6; cottonseed hulls, 21.0; defluorinated rock phosphate, 1.1; trace mineralized salt, 1.1; and vitamin (A and D) supplement. Trace mineralized salt was guaranteed to contain between 96.7 and 98.9% NaCl and not less than the following: (in %) Mn (manganous oxide), 0.200; Fe (ferrous carbonate), 0.160; Cu (copper oxide), 0.033; Co (cobalt oxide), 0.010; I (calcium iodate), 0.007; Zn (zinc oxide), 0.005. The vitamin supplement contained, per gram, 10,000 USP units vitamin A as palmitate in gelatin and 2,500 IU units of vitamin D as activated animal sterol and an unspecified amount of calcium carbonate and soybean meal rough as carrier. On a percentage basis, the analysis of this diet as fed was: crude protein, 15.4; ether extract, 1.4; acid detergent fiber, 15.6; ash, 5.0; NFE, 50.0; Ca, 0.5 and P, 0.6; and 0.9 ppm Ni.

The group average weights at the beginning of the preliminary period were 104, 105, 104, and 105 kg for the subsequent 0, 62.5, 250 and 1000 ppm nickel treatments, respectively. Body weights were obtained prior to and following the preliminary period, and at 2-week intervals thereafter. Careful clinical examinations were made by an attending veterinarian at least twice each week.

The first 12 animals (3 per treatment) completing the 8-week treatment period (from 13 to 21 weeks of age) were placed in collection crates for digestion and balance studies using a 5-day preliminary and a 5-day collection period. At this time and before the diets were changed these 12 animals were killed for histological studies. At the end of the 8-week treatment period the remaining animals were returned to the basal diet for a 6-week recovery period. When the animals were killed, sections of the liver, kidney and testis as well as rumen, abomasum and duodenum were studied histologically.

Volatile fatty acids of rumen fluid were determined on samples collected when the animals were killed, using a gas chromatograph[5] equipped with a hydrogen flame ionization detector. The samples were acidified to pH 2 with 3.5% sulfuric acid and injected into a 1.5-m column containing 20% neopenthylglycolsuccinate and 2% phosphoric acid.

Data obtained from these experiments were subjected to an analysis of variance and standard errors were calculated from the pooled error mean square (20).

RESULTS AND DISCUSSION

Calves fed the 62.5 ppm Ni diet ate about the same amount of feed and gained at the same rate as controls (table 1). Feed consumption and weight gains were reduced by 13 and 11%, respectively, by the 250 ppm Ni diet, the effects being significant at the 20% probability level. The 1000 ppm Ni diet drastically reduced feed intake ($P < 0.001$) and resulted in weight losses by every animal during the 8-week treatment period. Animals receiving the 1000 ppm Ni diet exhibited an immediate aversion for it and no adaptation with time. Failure to maintain their weight did not result in severe emaciation of those fed 1000 ppm Ni but the animals appeared to stop growing (fig. 1). Outwardly the calves appeared normal in every respect and were judged to be younger animals

[5] Aerograph Hy-Fi Model 600-D, Varian Aerograph, Walnut Creek, Calif.

TABLE 1

Average weight, daily feed consumption and daily live weight gains of young dairy bulls fed nickel-supplemented diets for 8 weeks and during a 6-week recovery period when the basal diet was fed

	Level of nickel supplementation, ppm				SE[1]
	0	62.5	250	1000	
			kg		
Treatment period[2]					
Initial weight	113	110	114	111	
Feed consumption	5.58[a,3]	5.34[a]	4.87[a]	1.41[b]	0.34
Rate of gain	1.33[a]	1.35[a]	1.18[a]	−0.16[b]	0.10
Posttreatment period[4]					
Initial weight	186	165	176	99	
Feed consumption	7.81[c,5]	6.50[cd]	6.30[cd]	4.67[d]	0.55
Feed intake/100 kg avg wt	3.41	3.17	2.94	3.29	
Rate of gain	1.47[c]	1.37[c]	1.39[c]	1.51[c]	0.24

[1] Standard error of a treatment mean calculated from pooled error mean square in analysis of variance.
[2] Six animals per treatment (five on 62.5 ppm).
[3] Within each measurement, those values not followed by the same letter are significantly different at the 0.1% probability level.
[4] Three animals per treatment (two on 62.5 ppm).
[5] Within each measurement those values not followed by the same letter are significantly different at the 5% probability level.

by impartial observers who were highly familiar with normal calves of these types. The general appearance of these animals suggests the possibility of a relationship between nickel and growth hormones.

The growth data are plotted against nickel levels in figure 2. Interpolation from the graph indicates that 300 ppm Ni in the diet would have given a reduction in growth rate which was significant at the 5% level. Comparably, zero gain was estimated with a nickel level of 900 ppm.

During the 6-week recovery period, the animals that had received 1000 ppm Ni ate less total feed ($P < 0.05$) than controls but this effect is attributed to their smaller size as feed intake per unit of size was not lower (table 1). The growth suppression of the 1000 ppm Ni diet disappeared when the animals were fed the basal diet during the recovery period (table 1).

The eating pattern of those fed the different nickel levels was vastly different. Animals fed 250 ppm Ni ate small quantities throughout the 24-hour day. Those fed a 1000 ppm Ni diet ate small quantities immediately following the morning or evening feeding and ate only infrequently or not at all at other times.

The fresh weight of organs removed at time of slaughter did not differ greatly for animals fed 0, 62.5, or 250 ppm Ni but with the exception of heart, kidney, and brain were significantly lower for those given 1000 ppm Ni (table 2). Expressed as a percentage of the body weight when killed, the heart, kidney, and brain were larger in those fed 1000 ppm Ni while lung, spleen, and testis were not greatly affected by dietary treatment. In relative weight, liver and gall bladder were smaller in the 1000 ppm Ni animals (table 2).

Except for kidney, nickel appeared to exert little effect on any tissues histopathologically, and even in the kidney many of the observed aberrations were also found in the controls (table 3). As the level of nickel increased, one or more animals in each group were found to have more kidney damage. The condition appeared to be progressively more severe and culminated in pyelonephritis. Rumen, abomasum, and duodenum tissues were relatively unaffected by nickel treatment even though nickel has been reported to be astringent and to have muco-hemorrhagic properties (21). Since nickel had slight or no effect on the gastrointestinal tract, the reduced feed intake of those fed 1000 ppm Ni must be explained on another basis. A linear depression in feed intake has been shown with increasing nickel levels in a palatability study (22). However, since the calves receiving the 1000 ppm Ni diet appeared younger rather than severely emaciated as would have been expected on the basis of reduced feed intake only, it seems

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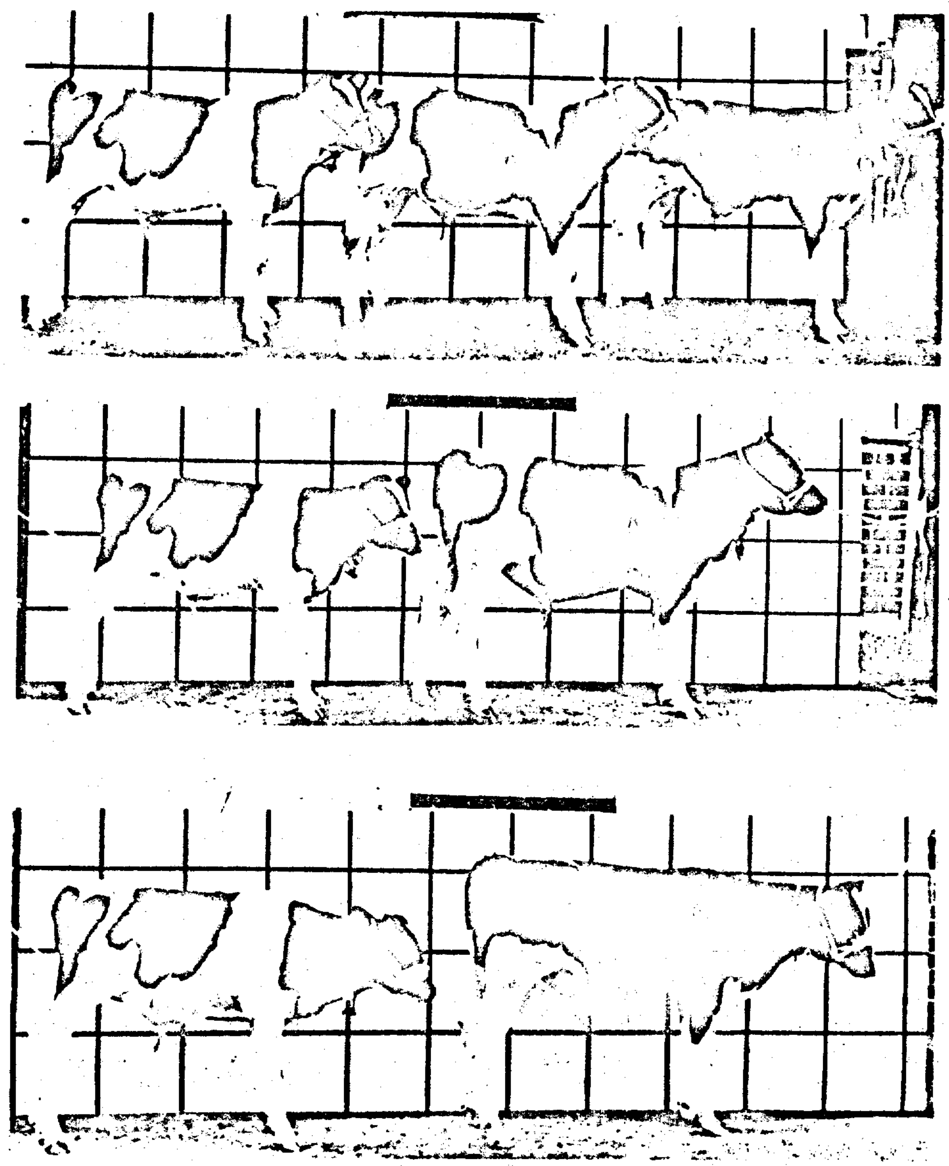

Fig. 1 At the top, 12-week-old male calves are shown at beginning of nickel treatment. From left to right: calf 48 (1000 ppm), calf 27 (62.5 ppm), and calf 14 (0 ppm). Liveweights were 110, 117 and 102 kg, respectively. Calf 48 (on left) and 27 are shown in the middle picture and calf 48 (on left) and 14 are shown at the bottom after 8 weeks of treatment. Live weight and average daily gain at end of treatments were 96 and −0.25, 188 and 1.27, and 175 and 1.30 kg, respectively, for calf 48, 27, and 14.

highly probable that a key mechanism in addition to reduced palatability is involved.

Data of digestion and balance trials conducted with three animals on each dietary treatment are presented in table 4. Apparent digestibility coefficients for dry matter, nitrogen, nitrogen-free extract and gross energy were not affected by any level

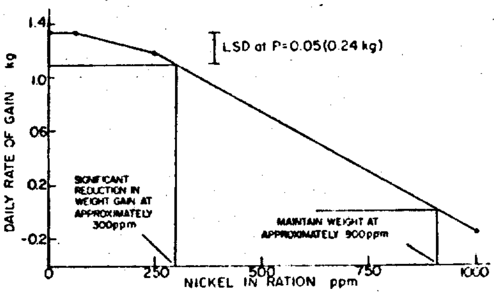

Fig. 2 Average daily rate of gain by male calves fed nickel at levels of 0, 62.5, 250 or 1000 ppm of total ration and estimation of nickel level that would result in statistically significant reductions in rate of gain or maintenance of initial weight (no gain). SE of a treatment mean was ± 0.10 kg with six animals per treatment (5 on 62.5 ppm).

of nickel supplementation. Nitrogen retention by calves fed 1000 ppm Ni was less than one-fourth that of the other three groups ($P < 0.05$) and reflects the reduced feed intake by this group. Both calcium and phosphorous retention were essentially equal for those fed 0, 62.5, and 250 ppm Ni but greatly reduced for the 1000 ppm group, probably reflecting the reduced feed intake of the higher nickel level. Expressed as a percentage of ingested calcium and phosphorus not recovered in feces, 58, 64, 61 and 60% of calcium and 66, 79, 76, and 87% of phosphorus was retained by the 0, 62.5, 250, and 1000 ppm nickel treatment groups, respectively.

The small positive nitrogen, calcium and phosphorus balances of the group fed

TABLE 2

Effect of nickelous carbonate on fresh weight of ruminant organs

Organ	Level of nickel supplementation, ppm				SE[1]
	0	62.5	250	1000	
			g		
Lung	2256[a,2] (1.13)[3]	2145[a] (1.05)	2233[a] (1.19)	1025[b] (1.01)	215
Heart	970[a] (0.49)	928[a] (0.46)	917[a] (0.49)	696[a] (0.69)	68
Spleen	506[a] (0.25)	456[a] (0.22)	445[a] (0.24)	207[b] (0.20)	32
Liver	3378[a] (1.70)	3421[a] (1.68)	3123[a] (1.66)	1305[b] (1.28)	218
Gallbladder	162[a] (0.08)	126[ac,4] (0.06)	137[a] (0.07)	38[b] (0.04)	20
Kidney	654[a] (0.33)	559[a] (0.27)	614[a] (0.33)	506[a] (0.50)	60
Testis	136[ac] (0.07)	157[a] (0.08)	156[a] (0.08)	80[b] (0.08)	13
Brain	382[a] (0.19)	345[a] (0.17)	366[a] (0.19)	319[a] (0.31)	14

[1] Standard error of a mean gross weight calculated from pooled error mean square in an analysis of variance, three animals per treatment.
[2] Within each measurement, those values not followed by the same letter are significantly different at the 1% probability level.
[3] Values in brackets are weights of organs as a percentage of body weight at time of killing.
[4] Any value followed by the superscript c is significantly larger than values at 1000 ppm Ni at the 5% probability level, all other differences are significant at the 1% probability level.

TABLE 3

Effect of nickelous carbonate on histopathological changes in selected tissues

Nickel suppl. in ppm	Animal no.	Tissue					
		Rumen	Abomasum	Duodenum	Liver	Kidney	Testis
0	1	N *	N	2	N	5	N
	2	1	1	N	3	6,7	N
	3	N	2	N	4	5	N
62.5	1	N	N	N	N	5	N
	2	N	N	8	N	6,9	N
	3	N	N	N	N	6,7	N
250	1	N	N	N	N	5,8,9	N
	2	N	N	N	N	5,10	N
	3	N	N	N	N	5	N
1000	1	N	N	N	N	5,6	N
	2	N	N	N	11	12	N
	3	1	1	N	3		N

* Explanation of code: N, Normal; 1, cellular infiltration; 2, gastroenteritis; 3, proliferation of bile duct epithelium; 4, focal microscopic abscesses; 5, glomeruli interstitial nephritis; 6, dilation of collecting tubules; 7, cellular infiltration of interstitial and glomeruli tissues; 8, lymphocytic infiltration with congestion; 9, congestion of medulla; 10; hyaline cast in collecting tubules; 11, extramedullary hematopoiesis; 12; pyelonephritis.

TABLE 4

Effect of nickelous carbonate on digestibility and retention of some dietary nutrients

Measurement	Level of nickel supplementation, ppm				SE[1]
	0	62.5	250	1000	
Apparent digestibility coefficient, %					
Dry matter	65.0	66.3	61.6	63.1	2.42
Nitrogen	66.7	69.5	67.6	73.0	2.79
NFE	80.6	80.3	78.6	80.9	3.06
Energy	64.4	65.0	59.3	62.0	2.43
Calcium[2]	58.2	64.2	61.4	60.4	
Phosphorus[2]	66.4	78.5	75.6	87.4	
Daily retention, g					
Nitrogen	52.5	45.1	50.1	11.9[3]	8.45
Calcium	15.3	16.9	16.4	3.3	7.32
Phosphorus	17.2	17.4	11.1	1.4	4.60

[1] Standard error of a treatment mean calculated from pooled error mean square in an analysis of variance, three animals per treatment.
[2] These values necessarily include both unabsorbed and reexcreted calcium and phosphorus but are indicative of the total metabolic status of these minerals.
[3] Significantly different from other treatments at $P < 0.05$. No other differences for any of the measures in this table were significant at the 5% level of probability.

TABLE 5

Effect of nickelous carbonate on rumen volatile fatty acids

Measurement	Level of nickel supplementation, ppm				SE[1]
	0	62.5	250	1000	
			molar %		
Acetate	57.8[2]	57.9	53.0	57.2	4.40
Propionate	29.9	25.8	37.9	35.1	4.95
iso-Butyrate	1.1	1.1	0.6	1.0	0.55
Butyrate	7.2	9.5	5.7	4.3	1.79
iso-Valerate	1.7	2.7	1.0	0.3	0.73
Valerate	1.9	2.7	1.6	1.7	0.83
Caproate	0.5	0.3	0.2	0.4	0.24
pH	6.4	6.4	6.3	6.2	
Total mmole/liter	133.5	149.3	146.0	115.4	

[1] Standard error of a mean calculated from pooled error mean square in an analysis of variance, three animals per treatment.
[2] Within each measurement, none of the values in this table were significantly different at the 5% level of probability.

1000 ppm Ni were not significantly different (5% probability level) from zero (table 4). Considered relative to the small weight loss (table 1) (also not significantly different from zero at 5% probability level), it appears that the calves may have been depositing limited amounts of proteinaceous and bony tissue which, weight-wise, was more than offset by loss of fat and other nonnitrogenous material. Since the animals were at an age when rapid growth of structural tissues is normal, such differential growth is not unexpected and because of the very limited amount of such growth would not greatly affect gross appearance.

Rumination of all animals was low with regurgitation infrequent. However, regurgitation appeared to be least frequent in animals fed the highest nickel level. This probably is due to the relatively fine particle size of the basal diet and by the greatly reduced feed intake of those fed 1000 ppm Ni. Reduction in rumination results in decreased salivary flow (23) which will affect rumen pH. Rumen protozoa are pH sensitive (24) and numbers of viable bacteria are higher in defaunated animals (25). A shift in the molar percentage of volatile fatty acids (VFA) in the rumen results from a combination of these factors (26). Data on rumen fluid VFA concentrations are shown in table 5. All animals had depressed acetate and butyrate and increased propionate when compared to normal values (26). The increase in propionate and decrease in butyrate were more pronounced at the higher levels of nickel feeding.

Average daily nickel intake was 1218 and 1410 mg for those fed the 250 and 1000 ppm Ni diets, respectively. The total amount of nickel ingested appears to exert little influence on animal performance. However, the rate of intake has a profound effect. Reduced feed intake and rate of gain, while appearing to be caused by animal rejection of feed containing high levels of nickel, may be a physiological response to nickel intake, rather than a simple palatability problem. Feed rejection continued over an 8-week period with little or no animal adaptation to feed. Thus it would be of interest to repeat this work over a longer period of time to ascertain the animal's ability to adapt to this feeding regimen and also to determine the long-range effects of high levels of nickel in bovine tissues.

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Journal Dairy Science
53(11)1545-48 (1970)

Effect of Nickel Supplementation on Production and Composition of Milk [1]

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Abstract

Three concentrations of nickel carbonate were fed to 3 groups of 5 lactating dairy cows each. Nickel carbonate was mixed in the concentrate ration at 0, 50 and 250 ppm of elemental nickel and concentrate fed at a ratio of 1kg per 3kg of milk produced. Average daily consumption of supplemental nickel per cow was 0, 365 and 1,835mg, respectively. No significant effect on milk production, milk composition, animal health or feed consumption was observed. Within the detectable limits of the analytical procedure, feeding nickel did not increase nickel in milk and never exceeded that in plant processed milk. None of the milk samples from cows fed 250 ppm nickel contained as much as 0.1 ppm nickel, which was the lower reliability limit of the procedure. Less than 0.12% of the supplemental nickel appeared in the milk.

Introduction

Knowledge relating to milk content of substances which are toxic or potentially toxic is highly desirable. Also a much better understanding of the principles which regulate the transfer of many trace substances from feed into milk is needed. Nickel is toxic at 40 to 60 ppm for plants (4,5), 700 ppm as the acetate or sulfate for chicks (13), 1,100 ppm as the acetate for mice (14), but rats tolerated up to 1,000 ppm as the carbonate or the metal without apparent toxicity (9,10). Generally plants contain less nickel (4,11), usually 0 to 5 ppm but often many times more (4). The normal nickel content in milk has not been established but conflicting reports have been published (2). Archibald (1) fed 145mg of elemental nickel daily as nickelous chloride to lactating dairy cows and failed to detect an increase of nickel in milk.

The objectives of this study were to ascertain the effect on animal performance and on nickel, fat, protein, and solids-not-fat of milk when lactating cows were fed nickel in amounts lower than those at which toxicity symptoms have been observed with monogastric animals (13,14) but greatly exceeding amounts used in a lactation experiment (1).

Experimental Procedures

Fifteen multiparous, lactating dairy cows with daily production between 16.5 and 30.0kg were approximately equalized into groups of 3 on age, weight, milk production and stage of lactation. These were randomly allotted to each of 3 treatments. Nickelous carbonate ($NiCO_3$) was added to the concentrate to provide 0, 50, and 250 ppm of nickel on an as-fed basis. Nickel carbonate is relatively insoluble in water but was determined to be readily soluble in rumen fluid.

One kilogram of concentrate was fed for each 3kg of milk produced and any concentrate not consumed after approximately 1 hour was weighed. The concentrate consisted of soybean meal, 25.0%; oats, 20.0%; shelled corn, 52.5%; defluorinated calcium phosphate, 1.5%; and trace mineralized salt, 1.0%. The trace mineralized salt was guaranteed to contain not less than: 0.228% Mn as manganous oxide, 0.160% Fe as ferrous carbonate, 0.033% Cu as copper oxide, 0.010% Co as cobalt oxide, 0.007% I as calcium iodate, 0.005% Zn as zinc oxide; 97.8 to 98.8% NaCl and technical white

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Received for publication March 24, 1970.

[1] Technical contribution no. 850, South Carolina Agricultural Experiment Station. Published by permission of the Director.

University of Georgia, College of Agricultural Experiment Stations, Journal paper no. 753, College Station, Athens.

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[4] Agricultural Chemical Services, Clemson University.

mineral oil and iron oxide of unspecified amounts. All animals were fed corn silage ad libitum as one herd. On a dry matter basis, the concentrate contained 7.9% ash, 23.3% crude protein, 7.4% acid-detergent fiber, 3.1% ether extract, 0.78% calcium and 0.99% phosphorus. Corn silage, on a dry matter basis, analyzed 6.4% ash, 8.6% crude protein, 32.3% acid-detergent fiber, 2.0% ether extract, 0.24% calcium and 0.17% phosphorus.

Every cow was examined and observed at least twice per week by an experienced veterinary clinician for indications of any abnormalities. Milk weights were recorded daily, and a 24-hour sample was taken from each animal at the end of a 1-week preliminary, at 2-week intervals for 6 weeks during treatment, and at the end of 1-week post-treatment. Precautions were taken to prevent the milk from contacting any metal. The interior of the milk claw, the milk cock and underside of the milk pail cover were coated with an acrylic resin. After the walls of the pail had been moistened with water, a .008cm polyethylene bag was inserted and the top folded around the pail opening and held in place by rubber restraints. The pail, with liner in place, was then slowly filled with water. This procedure forced trapped air to the top of the liner where it was evacuated by releasing the rubber restraints. With the trapped air evacuated, the polyethylene liner strongly adhered to the moistened side of the milk pail resisting being drawn by vacuum to the top of the pail. Samples of milk were removed from the pail by a "plastic" cup and were stored frozen in 1-liter polyethylene bottles.

Fifty milliliter samples, in duplicate, from each cow during both the pretreatment period and at the end of 6 weeks' treatment were transferred to 125ml Erlenmeyer flasks. Samples were dried in a vacuum oven and wet ashed with a sulfuric-perchloric-nitric (1:3:10) acid mixture. Reagent blanks containing the same quantity of acid mixture were prepared identically in duplicate and treated the same as the samples. The pH of the samples was adjusted to approximately 2.5 (meta cresol purple indicator, pH range 1.2–2.8) by putting them in a desiccator containing a small amount of NH_4OH. The NH_3 evolved from the NH_4OH was absorbed by the acidified milk samples. When the samples reached the end point, they were transferred to Babcock milk test bottles. The nickel in the original 50ml of milk was complexed into a 5ml organic phase which was raised into the neck of the Babcock bottle with glass-distilled, deionized water. Aspiration was directly from this organic phase into an atomic absorption spectrophotometer[5] with a standard (10cm slot) burner head. A scale factor of 1 and slit width of 0.3mm were used. Range was the ultraviolet wavelength 2,320 A. A hollow cathode nickel lamp was used with lamp current at 25mamps. A stable flame was obtained with air flow at 6.0 and acetylene flow at 0.32kg/cm^2.

Milk was analyzed for fat by the method of Babcock (3), for protein by dye binding procedure (12), and for solids-not-fat by the Mojonnier method (7).

Results and Discussion

No abnormality was observed in any cow during the experiment. In Table 1 the animal performance data for the 3 treatments are presented. Concentrate consumption was comparable for each of the 3 treatment groups indicating no effect of nickel. Likewise, milk production, milk fat, solids-not-fat, and protein were unaffected by the treatments. Average daily milk production during the treatments with 0, 50 and 250 ppm groups, were 93, 93 and 97% of production during the 1 week period before nickel supplementation. After adjustment by covariance for differences between animals within a group during the pretreatment standardization period, average milk fat tests were 0.20, 0.30 and 0.11% higher for the 0, 50, and 250 ppm nickel groups during the treatments. Daily intakes of supplemental nickel up to 1.8g exerted no statistically significant influence on concentrate intake, milk production, or milk composition. In earlier work nickel concentrations of 2 and 4 times that in this study rendered the feed much less palatable to dairy calves (8).

The lower limit of reliable detectability of the nickel method was 0.1 ppm. Only 6 samples contained as much as 0.1 ppm nickel; all of these were collected during pretreatment. The milk nickel contents (Table 2) are of about the same magnitude as reported by Archibald (1), who concluded that the detected nickel resulted from contamination. Mean nickel contents were identical for each treatment and equal to or less than basal values. If they were due to contamination, in spite of rigorous precautionary measures, it would indicate that the measures unintentionally improved with time and that very little nickel is secreted into

[5] Model 303, Perkin-Elmer Corporation, Norwalk, Conn.

TABLE 1. Effect of supplemental nickel in concentrate on cow performance.[a]

	Supplemental nickel, ppm			
	0	50	250	SE[b]
Concentrate fed (kg/cow per day)	7.76[c]	7.76	7.76	
Concentrate refused (kg/cow per day)	0.45	0.46	0.41	
Concentrate consumed (kg/cow per day)	7.31	7.30	7.35	
Supplemental nickel consumed (mg/cow per day)	......	365	1,835	
Body weight change (kg/cow per day)	0.67	0.83	0.83	
Milk produced				
Preliminary period (kg/cow per day)	24.2	23.8	23.4	
Treatment period (kg/cow per day)[d]	22.0	22.0	23.1	0.68
Fat in milk				
Preliminary period (%)	3.60	3.36	3.52	
Treatment period (%)[d]	3.80	3.66	3.63	0.09
Protein in milk				
Preliminary period (%)	3.85	3.87	3.75	
Treatment period (%)[d]	3.95	3.90	3.85	0.05
Solids-not-fat in milk				
Preliminary period (%)	8.75	8.70	8.48	
Treatment period (%)[d]	8.63	8.55	8.46	0.10

[a] Average values for 5 cows per treatment.
[b] Standard error of a treatment mean.
[c] None of the measures in this table were significant at the 5% level of probability.
[d] Adjusted by covariance for differences in milk and milk components during standardization period.

milk by the cow. However, nickel, like cadmium (6), is not increased significantly in milk by feeding large supplemental amounts. During the last treatment week, the average nickel intake was 1,792mg for cows fed 250 ppm nickel in the concentrate. None of the samples contained as much as 0.1 ppm, the reliability limit of the method. A concentration of 0.1 ppm would represent the transfer of 2.1mg of nickel into the milk; thus none of the analyzed milk samples of this group of animals could have contained a quantity of nickel that would have been present had 0.12% of the supplemental nickel been secreted into the milk.

While our data failed to define more exactly the nickel content of milk, they add to an understanding of this subject. Milk from cows fed high nickel supplements contained no more (< 0.1 ppm) and probably contains less nickel

TABLE 2. Nickel in milk from cows fed supplemental nickel in concentrate.

Animal number	Nickel supplementation in concentrate					
	0 ppm		50 ppm		250 ppm	
	Basal[a]	Treatment[b]	Basal	Treatment	Basal	Treatment
	(ppm)					
1	0.03	0.03	0.11	0.01	0.01	0.05
2	0.07	0.00	0.19	0.01	0.02	0.01
3	0.00	0.04	0.12	0.02	0.08	0.03
4	0.15	0.03	0.09	0.02	0.00	0.00
5	0.15	0.02	0.20	0.05	0.00	0.00
Average	0.08	0.02	0.14	0.02	0.02	0.02

[a] Basal, concentrate without supplemental nickel.
[b] Treatment, concentrate with supplemental nickel.

than milk processed through stainless steel equipment in any dairy plant. Two samples of pasteurized, homogenized milk from a vending machine were analyzed for nickel. Neither contained as much as 0.1 ppm nickel.

Nickel in concentrate at 250 ppm greatly exceeds the amount that a lactating cow would consume in feeds under any conceivable normal circumstances. Thus it is most unlikely that nickel in the cow's diet would add measurably to the nickel content of milk.

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Journal Sci. and Indust. Res. 9B(3) 70-76 (1950)

Toxicity of Nickel*

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The toxicity of nickel was tested using albino rats and monkeys as experimental animals and nickel carbonate, nickel soaps of mixed acids of refined arachis oil, and nickel catalyst suspended in oil and supported on kieselguhr as test materials. The dosage tried was 100 mg., 50 mg. and 25 mg. of adequate ration. Growth rate and reproduction performance were not significantly affected and no toxicity symptoms were observed in rats even after 3 to 4 months of continuous feeding. The retention of nickel for the 3 nickel preparations was highest with nickel carbonate. Appreciable amounts of nickel were found in the intestines and excreted in faeces. Adult monkeys maintained their weight and were in perfect health after 6 months of feeding on nickel-containing diets.

NICKEL does not, so far as is known, play any part in human or animal nutrition or in plant growth and no diseases of plants or animals have been attributed to the lack of it. Its interest to the nutrition worker arises from its frequent use as the pure metal, as nickel plate, or as a constituent of alloys in the construction of plant and vessels in food industries and for cooking and also as a catalyst in the hydrogenation of edible oils.

Nickel is found in some plants and animal organs as a trace element. Vegetables[1] like lettuce, cabbage, spinach and peas contain from 1·5 to 3·0 p.p.m., wheat 0·35 and fruits 0·15 to 0·25 p.p.m. In potatoes about 0·25 p.p.m. is found. According to Ramage[2] nickel occurs in a number of spices and herbal drugs and also is present in tea.

Some nickel is likely to be dissolved if strongly acid food is cooked in nickel or nickel-plated vessels but no poisoning from such usage has been reported owing probably to the fact that nickel salts are little absorbed or because the quantity thus absorbed is too small. Lehmann[3] found that food cooked in nickel utensils took up from 10 to 58 p.p.m. and he calculated that if nickel-ware was used exclusively in the kitchen, the total amount of nickel ingested daily would be about 117 mg. Cox[4] states that a beverage brewed in nickel contained 60 p.p.m.

According to Arnold[5], a daily dose of 80 mg. of nickel in the form of nickel pectinate given to young rats, corresponding to 1·25 gm. per kilogram of body weight, did not affect their rate of growth over a period of 8 weeks. Flinn and Inouye[6] found that nickel was excreted to the extent of 98 to 99 per cent in faeces. On the other hand, Kent and McCance[7] found in an experiment on the normal men with a nickel intake of 0·3 to 0·5 mg. daily that roughly two-third of the nickel was excreted in the urine and only one-third in the faeces. But their experiment did not show what would be the proportion at higher rates of intake.

According to Dzerzgowsky[8], doses of 10 to 20 mg. of nickel per kilogram of body weight causes no toxic symptoms in dogs, but 22 to 44 mg. per kilogram produces vomiting and diarrhoea. Injection of nickel salts in animals gives rise to toxic symptoms[9]. It is stated that Offerdahal[10] in 1913 took 0·5 gm. of powdered metallic nickel daily for a month without any ill effects.

The present problem arose out of the fear in the public mind that the use of nickel catalyst in the hydrogenation of oils was likely to leave small amounts of this metal in the finished product and that ingestion of the latter over prolonged periods might give rise to nickel poisoning.

In refined hydrogenated oil, the amount of nickel should be considerably less than one part per million[11, 12]. In India over a 100 samples have been recently analysed by Banerjee[13] and the highest value found in any sample was 0·4 p.p.m.

It was, however, considered worth while to test the effect of nickel administered to

* An enquiry under the *Council of Scientific & Industrial Research.*

young rats and monkeys in the form of nickel salts or colloidal nickel in fairly large doses in order to find out if toxic effects could be elicited after continued ingestion for prolonged periods.

The small amount of nickel in the finished hydrogenated oil when present is mainly in the form of finely divided metal, e.g. nickel catalyst. But there may be a possibility of formation of nickel soaps by interaction of free fatty acids with metallic nickel if the oil has not been carefully refined before hydrogenation. On the other hand, the presence of nickel in the ingested hydrogenated fat would render the possibility of nickel salt and soap formation during the process of digestion not altogether remote. Therefore, experiments were undertaken to test the effect of feeding to rats and monkeys nickel catalyst, nickel soaps and nickel carbonate.

Nickel Catalyst — This was finely divided nickel suspended in vegetable oil and supported on kieselguhr. It was kindly supplied by the *Hindustan Vanaspati Manufacturing Co. Ltd.* Bombay, on request. The nickel content of the catalyst was determined and found to be 19·17 per cent. Ash content was 40·86 per cent.

Nickel Soaps — These soaps were prepared by neutralizing with nickel carbonate the mixed fatty acids obtained on saponification of refined groundnut oil. The nickel content of these soaps was determined and found to be 10·20 per cent, calculated value being 9·7 per cent.

Nickel Carbonate — This salt was used for comparison with the nickel catalyst and nickel soaps.

The doses of nickel preparations were adjusted at three levels, 100 mg., 50 mg. and 25 mg. of nickel per 100 gm. of basal diet.

Experiments on Rats

The composition of the diet given to rats was as follows:

Whole wheat flour ...	58 parts
Bengal gram (without husk)	20 parts
Skim milk powder ...	10 parts
Vegetable fat (hydrogenated oil, m.p. 37°C.)	10 parts
Marmite	2 parts
Vitamin A ...	70 I.U./rat/day*
Vitamin D ...	13 I.U./rat/day*

* In weekly doses.

A week's supply of diet was prepared at one time and stored in the refrigerator. That nickel was evenly distributed in the diet mixture is shown by the following sample analysis (TABLE I).

TABLE I — NICKEL IN DIETS

	WEIGHT OF DIET TAKEN FOR ESTIMATION gm.	NICKEL EXPECTED mg.	NICKEL OBTAINED mg.
Group I	3·438	3·44	3·42
	4·213	4·21	4·22
Group II	5·684	2·84	2·85
	6·002	3·00	3·00
Group III	6·080	1·52	1·50
	8·432	2·11	2·12

Estimation of Nickel — Nickel was estimated in the diets, excreta and tissues colorimetrically using dimethylglyoxime[14] as the reagent. In the early stages of the investigation nickel was estimated gravimetrically as NiO to check the accuracy of the colorimetric methods. Having satisfied ourselves regarding the reliability of the latter, no further gravimetric estimations were done. In estimating nickel in the tissues, care was taken to remove completely the interfering substances such as copper, iron and phosphate. The colour obtained with the unknown was read off in a photoelectric colorimeter and results calculated by comparison with a standard curve prepared from solution made from pure nickel sulphate.

Feeding with Nickel Carbonate — 32 young rats, four weeks of age, were divided into four groups of eight each, males and females being equally represented in each group; one of these groups served as control.

The diets were mixed with a small quantity of water, steamed for 15 min. and given *ad lib* and so was water. Weights of the rats were recorded every week for eight weeks. The weekly average weights for each group are given in Table II.

Feeding with Nickel Soaps & Catalyst — Groups of young rats (eight in each group) of the same age as in the previous experiment were kept on the basal diet in which nickel soaps and nickel catalyst respectively were incorporated at levels similar to those used in the feeding experiments with nickel carbonate. Also a fresh control group was kept. This experiment was conducted in exactly the same way as the previous one.

The average growth rate for each group is given in Table III.

TABLE II — GROWTH OF RATS ON NICKEL CARBONATE-CONTAINING DIETS

Group & Ni content per 100 gm. diet	Average weights of eight rats in gm., Weeks								
	0	1	2	3	4	5	6	7	8
I — 100 mg.	43·0	58	66·7	76	85	92	86	109	115
II — 50 mg.	44·7	64	79·0	89	96	106	111	127	133
III — 25 mg.	43·0	65	79·8	92	99	109	113	129	135
Control	44·0	68	83·0	95	107	116	121	137	147

TABLE III — GROWTH OF RATS ON NICKEL SOAP & CATALYST

Group & Ni content per 100 gm. diet	Average weight per week in gm., Weeks								
	0	1	2	3	4	5	6	7	8
Nickel Soaps									
I — 100 mg.	40·6	59·8	68·5	78·6	86·5	96·5	101·0	109·6	112·9
II — 50 mg.	38·0	56·1	62·7	70·8	81·5	86·7	105·6	113·3	120·6
III — 25 mg.	38·1	59·7	62·6	73·7	85·1	102·0	107.0	112·8	123·2
Nickel Catalysts									
IV — 100 mg.	35·1	54·2	59·1	67·2	78·8	97·3	101·7	108·5	118·2
V — 50 mg.	34·1	55·8	60·1	73·6	85·1	103.0	106·0	118·6	125·2
VI — 25 mg.	36·0	59·4	67·8	78·8	93·7	107·0	116·2	125·4	128·5
Controls for groups I to VI	41·9	66·5	71·2	86·1	96·0	112·0	117·0	126·7	132·6

TABLE IV — METABOLISM OF NICKEL IN RATS

Group No. with mg. of Ni/100 mg. diet	Number of animals	Figures are for the whole of four day period				
		Food intake gm.	Ni intake mg.	Urine Ni mg.	Faeces Ni mg.	Retention Ni mg.
Nickel Carbonate						
I — 100	8	27·60	27·60	0·53	19·70	7·41
II — 50	8	39·73	19·87	0·24	15·18	4·45
III — 25	8	41·92	10·54	0·16	7·96	1·17
Nickel Soap						
I — 100	4	39·49	39·49	0·43	35·00	4·04
II — 50	4	37·72	18·86	0·22	16·25	2·39
III — 25	4	39·42	9·85	0·14	8·75	0·99
Nickel Catalyst						
IV — 100	4	38·35	38·35	0·30	34·75	3·30
V — 50	4	41·87	20·94	0·13	18·44	2·39
VI — 25	4	42·62	10·66	0·09	9·69	0·87

Food Intake of Rats — A weighed quantity of food was given daily to each animal; food left in the cage after 24 hr. was collected, dried and weighed. No appreciable differences in food intake were observed in the different groups.

Statistical analyses of the data contained in Tables II and III showed that the observed differences between the growth of controls and various other groups in all the three experiments were not significant. It can be concluded, therefore, that ingestion of nickel in the form of nickel carbonate, nickel soap or nickel catalyst at the fairly high levels tried in the experiment did not significantly affect the growth rates of the animals. The difference between the growth observed on 100 mg. dose of Ni as $NiCO_3$ and in controls appeared to be large, although the difference between the two groups was not statistically significant. The entire nickel carbonate experiment was repeated with fresh batch of rats, and the results confirmed the findings of the first experiment.

Metabolic Experiments — While the growth experiments were in progress, some of the animals were used for the study of nickel balances in the 5th and 6th weeks of feeding. The animals were placed in metabolic cages and after a preliminary period of three days, urine and faeces were collected over a period of four days. In the experiment with nickel carbonate, all the rats from each of the four groups were thus investigated, whereas from rats kept on nickel soap and catalyst diets, four rats from each group were studied. The average figures for intake and excretions are given in Table IV.

Distribution of Nickel in Tissues — From Table IV for metabolic data of nickel balances for rats, it will be clear that rats retained appreciable quantities of nickel on all the nickel-containing diets. Retention on the nickel carbonate diets has been larger than with the two other nickel preparations. This can be ascribed to the easy solubility of the compound in the stomach and hence easier absorption from the intestines of nickel when fed as nickel carbonate. The proportion of the ingested nickel found in faeces is the least in the nickel carbonate group. The excretion of nickel in urine is only slightly higher in this group than in the other two.

In view of the continuous retention of nickel albeit in small quantities, it was considered desirable to ascertain how the retained nickel was distributed among the different tissues of the body. After the growth experiments were over, i.e. in the ninth week of nickel feeding, two rats from each of the above groups were anaesthetized and blood drawn by heart puncture, the rats then being killed by excess of the anaesthetic. The following tissues were taken up for nickel determinations: femora, liver, kidney, spleen, heart, large and small intestines (together), testes and skin, in addition to the blood mentioned before. Nickel was determined colorimetrically after ashing and removal of interfering substances. The results are given in Table V.

Reproduction

After the observations on growth for eight weeks were over, males and females from the same group were paired. The object of this was twofold. The first was to find out if reproduction and lactation suffered from the inclusion of large amounts of nickel in the diet, and the second was to determine whether nickel retained in the body could be transferred by the mother to her offspring.

The rats kept for reproduction studies had been receiving nickel-containing diets for eight weeks previously. The same diets were continued so long as the rats were under observation throughout the period of gestation and lactation. Thus all these rats were being fed nickel-containing diets continuously for three to four months or more. The control rats were kept for corresponding periods on nickel-free diets.

Immediately after the young ones were born, two from each litter were weighed and

TABLE V — DISTRIBUTION OF NICKEL IN TISSUE

All the figures are for 100 gm. of fresh tissue weight

Group No. & mg. of Ni/100 gm. diet	Bones mg.	Liver mg.	Kidney mg.	Spleen mg.	Heart mg.	Intestine mg.	Testes mg.	Blood mg.	Skin mg.
Nickel Carbonate									
I — 100	35·85 34·69	1·11 1·30	4·31 3·37	2·86 3·31	5·01 4·00	2·54 2·56	1·86 2·68	3·10 2·11	1·25 0·71
II — 50	28·89 22·88	1·32 0·43	1·15 3·06	2·82 3·36	2·03 1·24	2·18 1·50	5·89 0·59	1·01 1·30	0·84 0·36
III — 25	18·76 13·52	0·10 0·15	1·19 1·00	0·55 0·26	0·79 0·76	1·84 0·69	1·32 2·33	0·48 0·34	0·24 0·10
Nickel Soaps									
I — 100	25·30 21·82	1·31 1·15	3·48 2·98	4·84 3·22	1·69 2·50	1·95 1·40	1·80 nil	1·06 1·17	0·78 0·78
II — 50	8·22 8·45	0·98 1·15	2·39 2·71	1·47 2·46	1·73 2·90	1·19 1·18	0·71 0·36	0·84 1·21	0·57 0·53
III — 25	3·60 5·33	0·42 0·57	1·44 2·02	0·92 1·65	0·88 0·91	0·64 0·74	nil nil	0·21 0·54	0·11 0·43
Nickel Catalyst									
IV — 100	14·73 17·56	0·89 1·27	2·55 2·37	3·47 1·85	1·79 2·29	1·08 1·28	0·22 0·55	0·81 1·00	0·56 0·65
V — 50	7·97 9·42	0·64 0·87	1·00 1·72	1·21 0·99	1·48 1·67	0·74 0·83	0·33 0·79	0·39 0·48	0·37 0·27
VI — 25	3·00 3·99	0·48 0·37	1·00 0·58	nil nil	1·03 0·63	0·51 0·34	0·92 nil	0·45 0·48	0·23 0·31
Control	nil nil	nil nil	nil nil	nil nil	nil nil	nil nil	nil nil	nil nil	nil nil

killed. The amount of nickel in the whole body was then determined. The results of these estimations are given in Table VI.

Experiments on Monkeys

Adult monkeys (*Macacus sinicus*) were fed on nickel-containing diets to confirm some of the findings obtained in rats with a different species of animals. The basal diet consisted of a mixture of 75 parts of whole wheat and 25 parts of Bengal gram (without husk) ground to flour. Nickel carbonate, nickel soaps of mixed fatty acids of groundnut oil and nickel catalyst were mixed with the combined flour to give 100, 50 and 25 mg. nickel respectively per 100 gm. of mixture. The prepared flour was made into chapatis and offered to the monkeys with small quantities of carrot and cabbage. The quantity of food given per monkey per day was 100 gm. In addition, small amounts of vitamin A and D were given orally per week. It was possible to use only 14 monkeys for this experiment; their distribution is shown in Table VII.

TABLE VI — NICKEL IN THE BODIES OF NEW-BORN RATS

GROUP NO. & MG. OF NI/100 GM. DIET	NO. OF YOUNG ONES IN LITTER	WEIGHT OF YOUNG ONES, gm.	NICKEL, MG./100 GM. BODY WEIGHT
NiCO$_3$			
I — 100	3	4·5	2·21
		4·3	3·00
	5	4·2	2·10
		4·3	2·78
II — 50	9	4·7	1·70
		4·7	1·37
	6	4·0	1·24
		3·7	1·02
III — 25	5	4·1	nil
		3·9	nil
	6	4·8	nil
		4·6	nil
Control	8	3·1	nil
		3·9	nil
	10	4·0	nil
		4·3	nil
Nickel Soap			
I — 100	6	3·7	nil
		3·6	nil
	4	4·0	0·12
		4·1	0·18
II — 50	5	4·4	nil
		4·5	nil
	7	3·9	nil
		3·9	nil
III — 25	5	4·7	nil
		4·9	nil
	5	4·3	nil
		4·4	nil
Nickel Catalyst			
IV — 100	5	4·6	0·44
		4·6	0·33
	7	4·2	0·12
		4·3	0·15
V — 50	8	4·2	nil
		4·2	nil
	8	4·5	nil
		4·5	nil
VI — 25	7	3·9	nil
		4·0	nil
	8	4·3	nil
		4·3	nil
Control	9	4·9	nil
		5·0	nil

TABLE VII — DISTRIBUTION OF MONKEYS ON NICKEL-CONTAINING DIETS

LEVEL OF NI, MG./100 GM. FOOD	DIETS CONTAINING		
	NICKEL CARBONATE	NICKEL SOAP	NICKEL CATALYST
	No. of animals		
100	2	...	...
50	2	2	2
25	2	2	...
Nil	Two animals served as controls		

The feeding was continued for twenty-four weeks and weights were recorded weekly. As all the monkeys were adults, maintenance of weight together with the general behaviour and other manifestations of toxicity were used as criteria for the ill effects of nickel feeding.

During the experiment two monkeys died, one from the nickel soap group (23rd week) and one control animal (soon after 24 weeks were over). Post-mortem examination did not reveal the cause of death. Blood of the animals was examined only at the termination of the experiment. The initial and final weights of the monkeys and the results of blood examination are given in Table VIII. All the monkeys appeared in excellent health as judged by their alertness and general behaviour. It will be clear that with the exception of one monkey who lost 0·5 lb. during six months, all others have maintained weight or showed a slight increase. Even in the two monkeys which died suddenly for no detectable reason, the weight had been steady till the week before death when last weights were taken. So far as blood was concerned, nickel feeding did not appear to have any ill effects. The various values given in columns 5 to 7 in Table VIII fall within the range for normal monkeys of the same species reported by Rao and Rao[15].

Summary

In view of the possibility of contamination with nickel of hydrogenated oils used for human consumption, experiments were undertaken to test the toxicity of nickel.

Albino rats and monkeys were used as experimental animals. Nickel carbonate,

TABLE VIII — BODY WEIGHTS & BLOOD FINDINGS IN MONKEYS KEPT ON NICKEL-CONTAINING DIETS

Group No. & mg. of Ni/100 gm. diet	Serial No. of monkeys	Weight in lb.		Blood findings at 6 months		
		Initial	At 24 weeks	R.B.C. mill/c.m.m.	Haemoglobin gm./100 c.c.	W.B.C. per c.m.m.
Nickel Carbonate						
I — 100	1	6·00	6·50	6·50	14·4	19,200
	2	6·25	7·25	6·93	12·7	16,800
II — 50	3	7·25	8·75	6·17	14·4	...
	4	6·50	7·25	6·90	16·6	13,200
III — 25	5	5·25	6·50	6·92	13·9	18,200
	6	5·25	6·50	6·25	13·6	14,800
Nickel Soap						
II — 50	7	6·00	6·00	6·53	16·9	18,200
	8	5·75	Died after 22 weeks (wt. 5·5)	...	...	...
III — 25	9	4·50	4·00	7·06	14·4	17,000
	10	5·25	5·25	6·51	14·8	20,400
Nickel Catalyst						
II — 50	11	4·50	5·25	6·68	13·6	15,650
	12	5·75	6·50	6·27	12·7	17,600
Control	C1	4·50	4·50	7·50	13·9	16,350
	C2	4·25	5·00	...	...	...

nickel soaps of mixed fatty acids prepared from refined groundnut oil and nickel catalyst suspended in oil and supported on kieselguhr were used as test materials. The dosage was fixed at three levels, viz. 100, 50 and 25 mg. nickel per 100 gm. of an adequate ration.

In rat experiments, growth, reproduction performance, metabolism of nickel, nickel content of tissues and of the offspring were studied.

In experiments on adult monkeys, maintenance of weight, effect on health and on blood picture were observed, during and after continuous feeding for six months on nickel-containing diets.

Results of Rat Experiments — (1) There were no significant differences in the growth rate (over eight weeks) of rats on nickel-containing diets as compared with that of control animals. The reproduction performance was also similar in the respective groups. The general condition of the surviving rats, fed on nickel diets for three to four months, was indistinguishable from that of the controls.

(2) The retention of nickel from the three nickel preparations tested was found to be highest on nickel carbonate. No appreciable difference between the other two groups were observed.

(a) Approximately 71 to 91 per cent of ingested nickel was found in the faeces. On nickel soap and catalyst, the average faecal nickel amounted to 87·9 and 89·9 per cent respectively, whereas on nickel carbonate it was distinctly lower, i.e. 74·4 per cent.

(b) The percentage of ingested nickel excreted in urine also varied with the nickel preparation; with nickel carbonate 1·56 per cent, with nickel soaps 1·21 per cent and with nickel catalyst 0·77 per cent.

(c) Within each group, the level of nickel intake did not make any appreciable difference in the proportions excreted in faeces and urine respectively.

(3) On the three nickel diets, appreciable amounts of nickel were found in the tissues. As arranged in order of decreasing nickel content, they would be, bone, spleen, kidney, heart, intestine, blood and testes. The nickel content of tissues of rats on nickel carbonate was higher than in the other two groups for corresponding levels of nickel intake.

(4) At the highest level of intake, i.e. 100 mg. per 100 gm. ration, nickel was found in the body of one-day old young ones. Those born of mothers on nickel carbonate showed the highest nickel content. At 25 mg. per 100 gm. ration, no nickel could be detected in the bodies of the young ones from mothers in all the three groups.

The work carried out thus far shows that on the comparatively high doses of nickel tested in rats, no toxic symptoms could be elicited even after three to four months of continuous feeding.

Results of Monkey Experiments — At the levels of nickel intake tested over a period of six months, adult monkeys maintained their weight and at the end were in perfect health. The haemoglobin, R.B.C. and W.B.C. counts of blood were normal in monkeys fed on nickel-containing diets.

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J. Nutrition 3: 141-156 (1930)

DOES THE NICKEL DISSOLVED FROM THE CONTAINER DURING PASTEURIZATION CATALYZE THE DESTRUCTION OF THE VITAMINS OF MILK?

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Received for Publication—January 6, 1930

DRINKER, *et al.*, (1) state that Geerkens in 1883 recovered 0.022 gram metallic nickel from two liters of milk that had stood eight days at room temperature in a nickel container. This amounts to 11 parts per million. According to Donauer (2) sweet milk at room temperature dissolves only one-tenth as much nickel as at the pasteurization temperature. On standing for eight days at room temperature milk would develop high acidity and it would seem that more than 11 parts per million should be dissolved since solutions of greater acidity dissolve nickel faster than neutral or alkaline solutions. Drinker points out that analyses of small amounts of nickel made before the development of the colorimetric method utilizing potassium di-thio oxalate are open to question. Donauer states that sweet milk at the pasteurization temperature dissolves 0.07 mg. of nickel per sq. cm. of surface exposed in 24 hours.

A most thorough review of the literature by Drinker *et al.* (1), concerning the occurrence, biologic and physiologic action, and toxicology of nickel and its various compounds, fails to shed any light upon its ability to catalyze the destruction of vitamins. The use of nickel as a catalyst in the hydrogenation of oils suggests that it might also catalyze the destruction of vitamins during the pasteurization of milk. Furthermore, the work of Hess (3) suggested, and the later work of Hess and Weinstock (4) indicated, a catalytic destruction of vitamin C in milk by copper. Since nickel is being used more extensively in industrial machinery, and especially in dairy manufacturing equipment, it seemed important to investigate the possibility of such a destruction. If only a comparatively small per cent of the vitamin content of milk or other foods were destroyed by such a catalysis, this would in the aggregate be of very great importance in human nutriton.

Experimental

Source and Quality of Milk. Certified milk averaging better than 4 per cent fat was obtained iced from a high grade farm near Rochester and was stored in a refrigerator at 34° F until pasteurized and, following pasteurization, until fed. The herd from which this milk was obtained was composed of Holsteins and Guernseys in all stages of lactation and was fed on a modern dairy ration of silage, hay, and grain. In summer the cows were upon pasture supplemented by grain. The night's milk was cooled and stored in a tinned copper holding vat (inspected to see that no copper was exposed) until the morning's milk was cooled and added, resulting in a milk of remarkably unifom composition. Most of the variations common to the milk of an individual cow were eliminated by pooling the daily production of a large herd.

Pasteurization of Milk. A container of *Berndorf-rein* nickel 16 cm. high and 12 cm. in diameter and a Pyrex beaker of approximately the same dimensions were filled with milk, placed on ringstands in a hot water bath, heated by gas plates to the pasteurization temperature (145° F), and held for 30 minutes. The milk in the two containers was stirred continuously during heating by motor-driven glass rods, both at the same speed.

Because of the greater specific heat and conductivity of nickel, it was necessary to heat the milk in the Pyrex beaker to 120° F before placing the nickel container in the water bath. Both would then reach 145° F at nearly the same time. Care was taken to keep the surface free from the coagulum, which forms at the liquid-air line, to approximate the conditions in commercial pasteurization. In this way full opportunity was given for oxidation to proceed if such a reaction should occur.

Nickel Content of Milk. A sample of this milk, pasteurized in glass, when analyzed by the method of Fairhall (1) showed no nickel, while milk pasteurized in nickel showed 15 parts of nickel per million. A second pasteurization in the same container without washing caused solution of 18 parts nickel per million or 18 mg. Ni per liter of milk.

At the former rate 0.0038 mg. nickel was dissolved per sq. cm. in thirty minutes. If solution had continued at this rate for 24 hours, which is improbable, 0.18 mg. per sq. cm. would have been dissolved, or more than twice that found by Donauer. At the end of a pasteurization period of thirty minutes the walls of the container were coated with a blackened precipitate of milk solids. The fact that during the second pasteurization 20 per cent more nickel was dissolved than during the first, indicates that in commercial milk plants when a pasteurizer is used continuously for a half day or even longer without washing, the rate of solution might be considerably greater than in this experiment.

For this container 12 cm. in diameter and 16 cm. high the ratio of cubical contents to surface exposed to the milk was 0.39 sq. cm. to 1 cc., while for a continuous flow pasteurizer in the 2 in. pipes in which heating occurs this ratio is 2 sq. cm. to 1 cc. and in the holding pipes of 5¾ in. diameter the ratio is 1.44 sq. cm. to 1 cc. For the holding type of pasteurizer of 300 gallons capacity the ratio is as low as 0.11 sq. cm. to 1 cc. Milk pasteurized by the continuous flow method would be subject to greater exposure than in the container used in this experment, while in a large vat with revolving coils, the unit of surface exposed per unit of milk would be one-third that in our container.

Since *Berndorf-rein* nickel is more soluble in organic acid than rolled, drawn, cast, or electrolytic nickel, it seemed that full opportunity was being afforded for solution and subsequent catalysis to occur during the pasteurization process if such action occurred under commercial conditions.

The nickel container before use for pasteurization purposes had a well-finished surface, perfectly smooth to the eye except for the marks left by the spinning process. After it had been used once or twice a week for pasteurization for twenty months, there were pits uniformly distributed over the surface which were plainly visible to the unaided eye, thus demonstrating the corrosive action of milk on nickel.

Vitamin A Experiments

Vitamin A Deficient Diet: For the investigation of the possible destruction of vitamin A by a catalytic action of nickel a basal diet of the following composition was used:

Commercial corn starch	76%
Casein (A Free)	18%
Agar agar	2%
Mineral salts (5)	4%

The casein was an Argentine product of very high quality purchased from Atterbury Brothers of New York. It was extracted continuously with hot alcohol for one week, dried, finely ground, and heated in an electric oven for one week at 95° to 100° C with stirring twice daily to assist in oxidation of any remaining traces of vitamin A. A feeding trial showed this to be free of the vitamin B complex according to present accepted Standards. The mixed ration was irradiated for thirty minutes with stirring at twenty inches from a quartz-mercury vapor lamp (Hanovia) to provide vitamin D.

Technique: Rats were obtained at approximately 3 weeks of age at 35 to 40 grams weight and placed in screened bottom cages to prevent access to feces. The basal A-free diet was weighed in twice weekly and the surplus weighed back at the succeeding feeding. The animals were fed 0.5

gram starch free yeast daily to supply vitamin B. They were also weighed twice weekly and weights charted on individual record cards.

Protective Method: A group of rats was obtained from the Albino Supply Company at 35 to 40 grams in weight and placed in groups of from 2 to 6 on each of the three kinds of milk (raw, pasteurized in glass, and pasteurized in nickel) on 5 cc., 10 cc., 15 cc., and 20 cc. levels of milk feeding. These rats were fed milk from the beginning of the experimental period. It was expected that even if there should be considerable destruction of vitamin A, the upper levels would still be high enough to provide for some growth. After twelve weeks of feeding there were no significant differences and therefore subsequent groups were placed on a 2 cc. level. The composite growth curves for these latter groups indicate that 2 cc. of milk do not provide sufficient vitamin A to provide for normal growth for more than 2 weeks but differences between the growth rates for the three groups were so small that they did not exceed the variation within the groups and so could not be considered significant. There were no external evidences of vitamin A deficiency other than lack of normal growth.

Curative Method. Preliminary Experiments: It was evident that if there was any destruction of vitamin A it was too small to be detected by the protective method. Accordingly a lot of rats from our own colony, weighing from 35 to 40 grams, was fed the basal A-free diet supplemented with 0.5 gm yeast daily until their body stores of vitamin A became depleted. They were kept on this ration until their weight became stationary for three weeks or fell below that of the third week previous. However, if xerophthalmia or diarrhea developed before they reached this weight, milk feeding was begun immediately. Five cubic centimeters of milk proved too high a level as recovery and growth were too rapid to reveal small differences. The rest of the animals were divided into three groups, for the three kinds of milk, and these groups in turn were subdivided and fed 1.0 cc., 1.5 cc., and 2.0 cc. respectively of milk. There were seven or eight animals in each group. The lower levels proved too low to cause rapid recovery and a steady increase in weight. The differences in weight between the animals on the 2 cc. level were neither sufficiently large nor uniform to be conclusive although there appeared to be an unfavorable effect due to nickel.

Definitive Experiments: Eighty rats averaging 33 grams in weight were purchased from the Albino Supply Company and depleted of their stores of vitamin A, as was the preliminary group.

While the preliminary group declined at six weeks at approximately 100 grams, this group did not decline until 8 weeks at 120 grams weight.

Those which declined first showed such a rapid fall in weight and such extreme symptoms that it seemed advisable to subdivide the group and begin milk feeding at once, as 2 cc. were not sufficient to cause recovery and restore the growth rate for animals of this size; consequently the groups as a whole do not show decline prior to milk feeding. The preliminary groups did not have equal average weights at the beginning of milk feeding, and this circumstance constituted an error for which no correction could be made. Sherman and Burtis (6) state that for vitamin A assay work only rats that do not differ greatly in weight should be used and advise a mini-

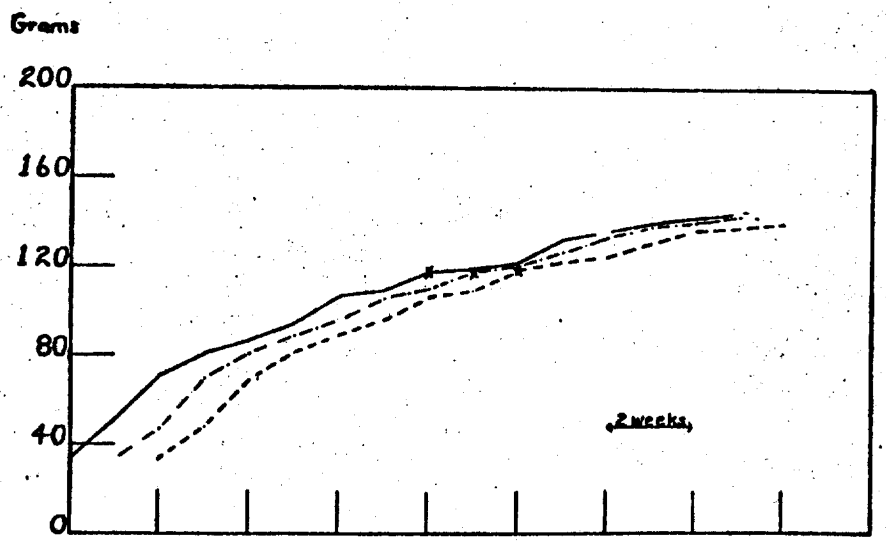

CHART 1.—Composite growth curves for rats fed raw milk, milk pasteurized in glass, and milk pasteurized in nickel for the cure of vitamin A deficiency.
Raw ——————— Pasteurized in Nickel- - - - - - - - - - -
Pasteurized in Glass -·-·-·-·-·-·-·-· X Began milk feeding.

mum weight of 70 to 75 grams and a maximum weight of 100 grams. They found with a limited allowance of vitamin A an inverse relationship between the body weight and the rate of gain.

The definitive groups were divided as evenly as possible with respect to litters, sex, and condition so that the average weight and deviation from the mean would be comparable for the three groups. Reference to Chart 1 will show that the composite growth curves for the three groups were almost identical up to that time. During the six weeks of milk feeding the average growth rate for the three groups was so nearly the same that there is no significant difference. Table I shows the average body weights for the three groups at significant age periods and Chart 1 the graphic record of their weights.

TABLE I
MEAN WEIGHTS OF RATS ON THE VITAMIN A EXPERIMENT

Group	No. Rats	Initial Weight	4 wks.	Beginning of milk feeding 8 wks.	3 wks. of milk feeding	6 wks. of milk feeding
		gms.	gms.	gms.	gms.	gms.
Raw	25	33.3	87.1	117.5	132.0	142.6
Past. in glass	25	32.9	89.4	118.5	133.1	142.9
Past. in nickel	25	33.0	88.5	118.9	131.6	139.9

During the period of milk feeding the most extreme variation between any two groups was 4 grams or approximately 3 per cent of the mean body weight, an amount that is well within the allowance for experimental error for biological work. Reference to Table II, a statistical analysis of the data for these groups, shows that the standard deviation in grams within the three groups at any time after milk feeding began was from two to three times the greatest difference in grams between the mean weights of the three groups. This proves quite conclusively that the average differences are not significant. Tables II and III follow:

TABLE II
VITAMIN A EXPERIMENT
STANDARD DEVIATIONS FROM MEAN WEIGHT

Kind of Milk	No. of animals	Initial Weighing	8th wk.	9th wk.	10th wk.	11th wk.	12th wk.	13th wk.	14th wk.
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Raw	25	3.4	11.0	11.1	10.5	11.3	11.6	11.7	12.2
Past. in glass	25	2.6	11.3	9.5	11.3	11.1	11.3	14.8	14.0
Past. in nickel	25	2.5	10.7	10.5	11.1	9.8	9.0	9.4	9.4

TABLE III
VITAMIN A EXPERIMENT
PERCENTAGE COEFFICIENTS OF VARIABILITY

Kind of Milk	No. of Animals	Initial weighing	8th wk.	9th wk.	10th wk.	11th wk.	12th wk.	13th wk.	14th wk.
Raw	25	10.2	9.3	9.4	8.6	8.5	8.5	8.3	8.5
Past. in glass	25	8.0	9.5	8.0	9.0	8.3	8.1	10.5	9.8
Past. in nickel	25	7.6	9.0	8.7	8.9	7.4	6.6	6.8	6.7

A study of the standard deviation and percentage coefficients of variability shows that the three groups were selected satisfactorily and that the data became more concordant as the feeding period progressed. The percentage coefficients of variability were smaller for the group receiving milk pasteurized in nickel than for the group receiving milk pasteurized in glass, and became increasingly more satisfactory as the end of the feeding period approached. The numbers of rats developing xerophthalmia and the average time required for recovery were also comparable for the three groups.

Table IV shows that the differences in consumption of basal diet and therefore in total food consumption, since the same amount of milk was fed each group, are negligible and indicates the comparative uniformity of the three groups.

TABLE IV
WEEKLY FOOD CONSUMPTION IN GRAMS

Groups	Raw	Past. Glass	Past. Nickel
Average of entire period	66.2	66.0	67.4
Average during milk feeding	66.3	66.6	67.8

The explanation for the fact that the preliminary groups began to decline at 100 gms. while the definitive groups did not reach this stage until 120 gms. lies in the difference of their source. Those used in the preliminary period were from our own stock while those in the latter experiment were from the Albino Supply Company and were fed a different ration, presumably higher in vitamin A content, resulting in greater vitamin A body stores. The observation of Sherman and Burtis relative to the existence of an inverse relationship between the body weight and rate of gain with a limited allowance of vitamin A was confirmed, especially by the rate of decline of the animals of the definitive group prior to milk feeding. They state further that males and females may be used interchangeably if the growth rate is approximately 3 grams per week. Since the gains were of this order and there seemed to be no consistent difference in the growth rate of the two sexes, they were averaged together to form composite curves.

The collective results of these experiments indicate no destruction of vitamin A by catalytic action during the pasteurization process unless it has been compensated by some favorable action now unknown.

Vitamin B Experiments

Vitamin B Deficient Diet: The basal diet was the same as that employed for the vitamin A experiments, with the exception that the casein was purified to remove vitamin B. This was accomplished by washing the casein continuously for a half week with 0.1 per cent acetic acid and for the remainder of the week with distilled water. The casein was then dried and finely ground. Control rats fed this diet plus daily additions of three drops of cod liver oil died in 14 days showing it to be very low in, if not entirely devoid of, vitamin B.

Technique: Rats were obtained from the Albino Supply Company at 3 weeks of age and placed in screen-bottom cages which prevented access to feces. They were fed basal diet *ad libitum* supplemented by 3 drops of cod liver oil daily in glass dishes to supply vitamins A and D.

Protective Method, Preliminary Experiments: Groups of four to six rats were placed upon 2 cc., 5cc., 8cc., and 10 cc. levels of milk feeding for each of the three kinds of milk. Those receiving milk on the 2 cc. and 5 cc. levels died too soon to manifest characteristic symptoms of polyneuritis. Those on the 8 cc. level made fairly rapid growth at first but later declined in weight and developed marked polyneuritis, while those on the 10 cc. level did not develop polyneuritis within eight weeks.

These animals received milk produced during the months of August and September which would contain less vitamin B than that produced in June when the vegetation was more luxuriant.

Definitive Experiment: A confirmatory group also secured from the Albino Supply Company at 41.9 grams was placed on the 8 cc. level the following March with 24 animals on each of the three kinds of milk. Table V gives the mean weights of the three groups at representative points throughout the feeding period and Chart 2 shows the composite growth curves for the three groups.

Table V
Mean Weights of Rats on the Vitamin B Experiment

Group	No. Rats	Initial	2 wks.	4 wks.	6 wks.	9 wks.
		gms.	gms.	gms.	gms.	gms.
Raw	24	42.3	69.5	72.0	74.9	74.0
Past. in glass	24	41.2	64.6	64.6	64.6	63.6
Past. in nickel	24	42.2	66.8	67.5	67.7	65.3

It will be noted that the group receiving raw milk made definitely greater and more uniform gain than the other two groups receiving pasteurized milk. These two groups follow the same general course and parallel each other so closely that the differences can not be considered significant. Of the 24 animals on raw milk 13 developed polyneuritis at an average of 55.5 days of milk feeding while the remaining 11 never developed even first symptoms. Of the group receiving milk pasteurized in glass, 23 developed first symptoms at an average of 51.7 days of milk feeding while of those receiving milk pasteurized in nickel 23 developed first symptoms at an average of 52.1 days of milk feeding. The two groups developed more

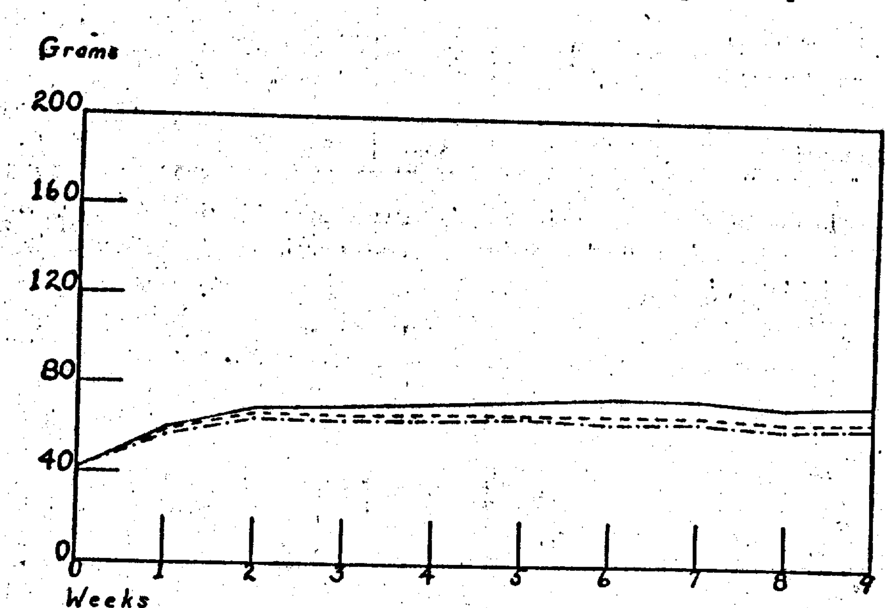

CHART 2.—Composite growth curves for rats fed milk, milk pasteurized in glass, and milk pasteurized in nickel as a source of vitamin B.
Raw ————————————
Pasteurized in Glass —·—·—·—·—·—·—·—
Pasteurized in Nickel — — — — — — — — — —

extreme polyneuritis terminating in a spastic condition without revealing any appreciable difference. Had there been any greater destruction due to the presence of nickel it could hardly have failed to be evident. Thus it will be seen that pasteurization destroyed part of the antineuritic factor (B) but that nickel did not increase this destruction. The development of dermatitis and the shedding of hair were evident in all three groups to the same extent, showing that there was no greater destruction of the pellagra-preventing factor (G) by contact with nickel.

The following statistical analysis (Table VI) shows that the body weights of the groups were satisfactory at the beginning of milk feeding. The two groups receiving pasteurized milk were almost equally concordant from the standpoint of standard deviation and also with respect to the percentage coefficient of variability as seen in Table VII.

TABLE VI
STANDARD DEVIATION IN GRAMS, VITAMIN B EXPERIMENT

Kind of Milk	No. of Animals	Initial Weight	3 wks.	5 wks.	8 wks.	9 wks.
Raw	24	8.1	10.0	11.2	11.3	10.9
Past. in glass	24	5.7	9.0	8.4	9.3	8.8
Past. in nickel	24	5.3	11.3	10.1	7.7	8.0

The rats of more extreme body weights were placed in the group receiving raw milk, a fact which explains the greater variability of this group at the initial weighing.

TABLE VII
PERCENTAGE COEFFICIENTS OF VARIABILITY, VITAMIN B EXPERIMENT

Kind of Milk	No. of Animals	Initial Weight	3 wks.	5 wks.	8 wks.	9 wks.
Raw	24	19.1	14.3	15.3	15.8	14.7
Past. in glass	24	13.8	14.0	12.7	15.0	13.8
Past. in nickel	24	12.5	17.0	15.0	11.9	12.2

The group receiving raw milk consumed an average of 29.7 grams basal diet per week while the groups receiving milk pasteurized in glass and milk pasteurized in nickel consumed 27.7 gms. and 27.1 gms. respectively. It is well known that limiting the intake of foods containing vitamin B lowers the food consumption. The lower food consumption of the groups receiving pasteurized milk is doubtless due to thermal destruction of a part of the antineuritic factor.

A study of growth rate, standard deviation, percentage coefficients of variability, and consumption of basal diet, fails to show an increased destruction of vitamin B due to the influence of nickel.

VITAMIN C EXPERIMENTS

Vitamin C Deficient Diet: The basal diet fed to the guinea pigs was that recommended by Sherman (7) and had the following composition:

Rolled oats	29.5
Wheat bran	29.5
Sodium chloride	1
Skim milk powder	30
Butter oil	10

The skim milk powder was prepared by the spray process by the Merrell-Soule Company and was heated in an electric oven at 95° to 100° C. for four hours to destroy all vitamin C.

Technique: Guinea pigs weighing approximately 250 grams were placed on the basal diet supplemented by 10 grams cabbage and 20 cc. milk

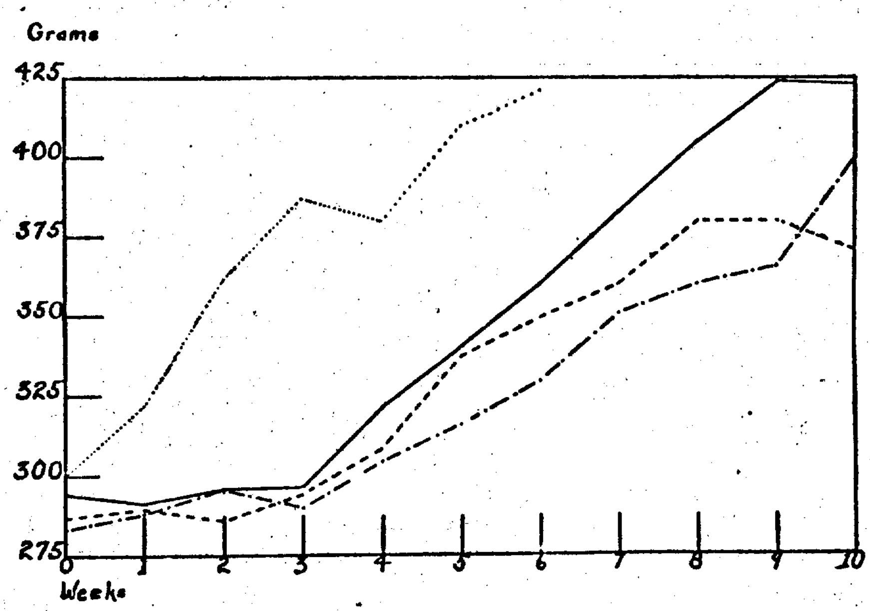

CHART 3.—Composite growth curves for guinea pigs fed raw milk, milk pasteurized in glass and milk pasteurized in nickel as a source of vitamin C. The control animal received 10 grams of cabbage daily as its source of vitamin C.
Raw ——————————— Normal ....
Pasteurized in Glass –·–·–·–·–·– Pasteurized in Nickel – – – – – – –

daily. When they reached 300 grams the cabbage was withdrawn leaving the milk as the sole source of vitamin C. The basal diet was fed *ad libitum*. All animals were divided between the three groups as evenly as possible with respect to size, sex, growth rate, and source. The three groups were fed simultaneously and from the same day's milk to eliminate any errors due to differences in weather, variations in milk or basal diet, or to variations in value of the vitamin supplements.

Weighings were made and charted biweekly.

Milk was fed in glazed porcelain dishes to avoid contact with metals.

Preliminary Experiments: Preliminary groups were started on the 20 cc., 30 cc., and 40 cc. levels of the three kinds of milk in an attempt to find a critical level for milk feeding. On the 20 cc. and 30 cc. levels there was an evident destruction of vitamin C due to pasteurization but no apparent difference between the two groups receiving pasteurized milk. On the 40 cc. level there was a much greater difference between the group receiving raw milk and those groups receiving pasteurized milk. On this level there did seem to be a significantly smaller gain made by the group receiving the milk pasteurized in nickel. However, since there were but seven animals on each kind of milk, the gain seemed too small to draw any conclusions in view of the large variations in growth rate of guinea pigs. This first lot was fed during late winter and early spring when the cows were upon winter feed.

Definitive Experiment. Some difficulty was experienced in the preliminary experiment in securing complete milk consumption at the onset of scurvy, so a definitive group of 72 animals was fed 20 cc. of summer milk to overcome this difficulty. Table VIII shows the mean weights while Chart 3 shows the composite growth curves for the groups on each of the three kinds of milk. It is evident that the group receiving raw milk made more satisfactory growth than the other two groups.

TABLE VIII
MEAN WEIGHTS OF GUINEA PIGS ON THE VITAMIN C EXPERIMENT

Group	No. of animals	Initial weight	4 wks.	6 wks.	8 wks.	10 wks.
		gms.	gms.	gms.	gms.	gms.
Raw	24	294	321	360	405	423
Past. in glass	24	283	304	329	360	399
Past. in nickel	24	287	308	349	380	371

The mean weights up to the eighth week indicate that the effect of nickel was not detrimental. The drop in weight at 10 weeks seems somewhat significant at first but a study of the individual data at this time shows it to be relatively unimportant. Of the group receiving raw milk 7 weighed more than 460 gms. while 2 weighed less than 300. Of those receiving milk pasteurized in glass 4 weighed over 460 grams and 5 under 300, while of those receiving milk pasteurized in nickel only one weighed over 460 grams and only one under 300 grams when they died of scurvy or when they were killed at 10 weeks. The small average drop of 9 grams (2 to 2.5 per cent of body weight) is much smaller than the average variation within either of the groups receiving pasteurized milk and so becomes insignificant. Also the number of animals in each group receiving pasteurized milk was reduced by death from scurvy to 16 at the eighth week and to 6 at the tenth week. The flatness of the three curves in Chart 3 for the first three weeks is due to the withdrawal at the beginning of the experimental period of the daily allowance of 10 gm. cabbage which was the only change in the ration at that time. Apparently the withdrawal of this bulky food necessitates a readjustment of the alimentary tract.

Reference to Chart 3 shows that pasteurization destroys part of the vitamin C of milk. The flatness of the growth curve also indicates that the raw milk was fed in amounts considerably below the optimum. The positive control was a typical animal fed the same basal diet but supplemented by 10 gm. cabbage daily. Up until 5 weeks of protective feeding there was no marked difference in the growth curves of the three groups. While the growth rates for guinea pigs are more variable than for rats, still with 24 animals on each group the composite curves should be comparatively reliable. One pig died of scurvy during the experiment from the group receiving raw milk while 7 died in the group receiving milk pasteurized in glass and 5 in the group receiving milk pasteurized in nickel. Consideration of the scorbutic symptoms of those animals dying before termination of the experiment shows no significant difference between the two groups receiving pasteurized milk. When the scorbutic symptoms of the animals that came to autopsy at the end of the feeding period are considered, there was no appreciable difference between the two groups receiving pasteurized milk. On an average the fragility of bone and occurrence of rosary were slightly greater for the group receiving milk pasteurized in glass, while the other symptoms were equally manifest. Those receiving raw milk exhibited decidedly less fragility of bone while the other symptoms showed approximately the same severity as manifested by the two groups receiving pasteurized milk. This must be due to the greater weight attained and the consequently greater amount of vitamin C required to maintain the animals in normal condition. The deficiency did not exist long enough in those receiving raw milk to produce fragility of bone but did exist long enough to produce more acute symptoms. The difference in the growth curves of the two groups receiving pasteurized milk from the fifth to the ninth week was approximately

6 per cent. This difference at first might seem significant but reference to Table IX of statistical data shows the percentage deviation from the mean to exceed this difference in growth. Inspection of this table shows that the group receiving raw milk was more satisfactory than the groups receiving pasteurized milk. The maximum increase in per cent deviation for this group is equal to the per cent difference in growth rate of the two groups receiving pasteurized milk as seen in Chart 3. However, the increase in per cent deviation for the latter groups exceeds this figure and shows it to be insignificant. The per cent deviation for the group receiving milk pasteurized in nickel becomes more concordant as the experiment progresses in marked constrast to the group receiving milk pasteurized in glass. In no way can these results be interpreted as showing a detrimental effect due to nickel.

TABLE IX
VITAMIN C EXPERIMENT
PER CENT DEVIATION FROM MEAN

Kind of milk	Initial weight	2nd wk.	4th wk.	6th wk.	8th wk.	10th wk.
Raw	7.4	9.5	12.5	13.9	12.3	12.0
Past. in glass	8.4	12.4	18.8	20.5	18.0	22.6
Past. in nickel	8.6	10.2	16.3	14.0	9.9	10.1

Hotchkiss (8) found that 0.65 gm. $NiCl_2$ (0.2925 gm. metallic Ni) per liter of culture media, equivalent to about 300 parts per million, was toxic to *B. coli*, that 0.13 gm. $NiCl_2$ (0.0585 gm. Ni) limited growth, and that 13 mg. $NiCl_2$ (5.85 mg. Ni) equivalent to 5 or 6 parts per million actually stimulated *B. coli* to greater growth. This indicates that nickel under some conditions plays a beneficial role in the metabolism of protoplasm. Hart (9) and coworkers and also McHargue *et al.* (10) have shown that copper assists iron in the regeneration of hemoglobin of anemic rats fed on an exclusive milk diet. Titus, Cave, and Hughes (11) have shown that manganese aids copper and iron in this same role. The fact that nickel is used as a catalyst in the hydrogenation of oils and that it induces a tallowy or metallic flavor (7) in butter made from cream pasteurized in nickel suggests that it might play a role similar to that ascribed to copper and manganese. It seems unlikely that nickel has catalyzed the destruction of vitamins in milk unless there has been a compensation due to the presence of nickel.

Summary and Conclusions

1. The nickel content of milk pasteurized in a nickel container was 15 parts nickel per million while the same milk pasteurized in a glass vessel contained no nickel.

2. A total of 390 rats and 140 guinea pigs was utilized for these experiments.

3. In testing the catalytic destruction by nickel of each of the vitamins A, B, and C, three groups of animals were fed a ration otherwise adequate and supplemented by raw milk, milk pasteurized in glass, and milk pasteurized in nickel respectively, to supply the vitamin in question.

4. There was no appreciable destruction of vitamin A by pasteurization in either a glass or a nickel container.

5. The antineuritic factor of the vitamin B complex was partially destroyed by pasteurization but there was no evidence of a catalysis of the destruction by nickel.

6. Vitamin C was partially destroyed by pasteurization but nickel did not seem to increase the destruction.

7. Determination of standard deviation and coefficients of variability shows the data to be satisfactory and to become more concordant as the experiment progressed. The differences between the two groups receiving milk pasteurized in glass and milk pasteurized in nickel are shown not to be of greater magnitude than the deviation within the groups.

8. Unless it can be demonstrated that nickel *per se* is beneficial to animals on these vitamin deficient diets, it is impossible to interpret the data as indicating any catalytic destruction of vitamin by nickel during the pasteurization process.

A study of the above summary and a study of the rate of development of deficiency diseases, acuteness of symptoms, autopsy findings, and growth curves, do not indicate that nickel dissolved from the container during pasteurization catalyzes the destruction of vitamins A, B, or C during the pasteurization process.

I wish to express my appreciation to Drs. J. R. Murlin, and H. A. Mattill for advice and guidance in the conduct of this investigation.

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Experimental Cell Research, **10**, *146–154* (1956)

STUDIES ON THE FUNCTION OF INTRACELLULAR RIBONUCLEASES

I. THE ACTION OF COBALT AND NICKEL ON *TETRAHYMENA PYRIFORMIS* W[1]

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Received May 20, 1955

Cobalt in various forms has many diverse effects in the animal organism and on animal and plant cells. Levy, Skutch and Schade [9] found that *Proteus vulgaris* placed in the presence of Co^{++} continued to synthesize ribonucleic acid (RNA) but not deoxyribonucleic acid (DNA) or protein. Miura and Nakamura [11] confirmed this action of Co^{++} using *Staphylococcus aureus* and *Escherichia coli*, and from studies on the action of Co^{++} on crystalline pancreatic ribonuclease (RNase) concluded that the inhibition of intracellular RNase accounted for the observed effects. Recently Heath [6] has reported that certain concentrations of Co^{++}, when added to actively growing chick heart fibroblasts *in vitro* cause abnormal mitoses in which the nucleoli persist throughout the whole cycle, and finally, with the formation of the new nuclei in telophase, are left in the cytoplasm of the daughter cells.

It would seem reasonable to explain the above effects of Co^{++} as being due to the inhibition of intracellular RNases by this ion. Inhibition of this enzyme (or enzymes) could conceivably result in the accumulation of RNA in the bacterial cells studied, or in the failure of the RNA containing nucleoli to be broken down during mitosis. As it has been reported [13] that another, quite different, RNase inhibitor heparin [14, 19] may also often cause the accumulation of RNA in cells in tissue culture, this would appear to be further support for the explanation first suggested by Miura and Nakamura. However, there are certain difficulties with this explanation which seemed to us to warrant further study. We have not been able to confirm Miura's and Nakamura's results [13] with regard to the action of Co^{++} and Ni^{++} on crystal-

[1] A portion of this material was presented as an abstract at the Seventh Annual Meeting of the Society of Protozoologists, East Lansing, Michigan, September 1955.

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line pancreatic RNase and furthermore, since this enzyme is apparently quite different from intracellular RNases [8, 12, 15], conclusions based on the effect of Co^{++} on the crystalline enzyme would not be valid. We have, therefore, investigated in some detail the effects of Co^{++} on the protozoan *Tetrahymena pyriformis* W. For comparison Ni^{++}, which is quite similar to Co^{++} in chemical properties but does not exhibit the same biological action at comparable concentrations, has been studied similarly. Experiments with crystalline pancreatic RNase have also been carried out.

MATERIALS AND METHODS

Tetrahymena pyriformis W was cultured in 2 per cent peptone containing 0.2 per cent Marmite (yeast preparation). For growth studies cells were grown in slanted test tubes containing 5.0 ml of medium. Optical densities were determined at appropriate intervals in a photoelectric colorimeter. Cobalt and nickel were determined by the method of Chilton [2]. When organic material was present, samples were ashed in air and the residue taken up in hot concentrated HCl. The Schneider procedure [17] was utilized to prepare a mixture of RNA and DNA nucleotides from cells, omitting extraction of lipids from the trichloroacetic acid (TCA) precipitate. DNA was measured by the method of Dische [3]. RNA was determined by difference after determination of the total absorption of the hot TCA extract at 260 mμ. RNase was determined by a modification, previously described [14], of the method of McCarty [10]. Nitrogen was estimated by a micro-Kjeldahl procedure. Crystalline pancreatic RNase was obtained from Worthington Biochemical Sales Co., Freehold, N.J.

RESULTS

In Vivo Effects of Co++ and Ni++

The effect of Co++ and Ni++ on the growth of Tetrahymena.—Cobalt and nickel as the sulfates were added to media in test tubes which were then autoclaved at 15 lbs for 20 minutes. Three test tubes for each concentration were prepared and one tube, which was uninoculated, served as a control. One loopful of 3 day W strain was transferred into the tubes which were then kept in the dark at room temperature in a slanted position. Tubes containing more than 1×10^{-4} *M* Co^{++} turned darker with time, possibly due to reaction of Co^{++} with histidine and oxygen in the medium [5]. The amount of Co^{++} and Ni^{++} originally present in the medium was less than 0.1γ per ml while there was 0.2γ of Cu^{++}. The results of the growth experiments are shown in Figs. 1 and 2. Co^{++} at all levels used inhibited the initial growth rate, but with concentrations ranging from 5.9 to 17.7γ per ml the final growth was greater than the controls. A concentration of 23.6γ per ml greatly increased the lag period and reduced the final growth at 216 hours to one-third that of the controls. There was no growth in tubes containing higher concentrations of Co^{++}. These higher concentrations (up to 47.2γ per ml) were not lethal to the cells, for the original inoculum remained viable for at least 240 hours. In another experiment Co^{++} was added to a culture of

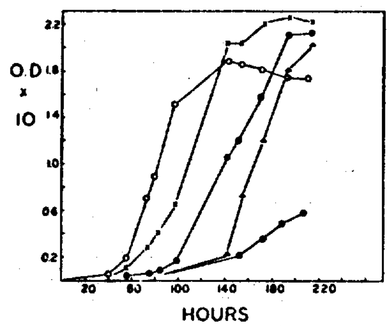

Fig. 1. The effect of Co^{++} on the growth of *Tetrahymena pyriformis* W in peptone medium.

○——○ Controls
×——× 5.9 γ Co^{++} per ml
●——● 11.8 γ „ „ „
△——△ 17.7 γ „ „ „
◓——◓ 23.6 γ „ „ „

rapidly growing *Tetrahymena* to give a concentration of 84γ per ml. The culture of approximately 10^5 cells per ml contained many cells in division. Three hours after adding the Co^{++}, as far as could be determined by microscopic inspection all of the cells remained viable but there were no further divisions.

Slater [18] has determined that *Tetrahymena pyriformis* E has a nutritional requirement for Co^{++}. He examined the effect of addition of Co^{++}, up to a maximum of 8γ per ml, to a Co^{++}-free synthetic medium and reported that as little as 0.1γ Co^{++} per ml gave good growth, while higher concentrations (4 and 8γ per ml), in agreement with the present results, slowed the initial growth rate, but gave a final higher population density.

The effect of Ni^{++} on the growth of *Tetrahymena* is depicted in Fig. 2. Concentrations up to 17.7γ per ml had little significant effect on the growth rate. In the range from 17.7 to 41.3γ per ml there was an increasing lag in the initial growth but after about 120 hours the growth in the tubes containing this range of concentrations somewhat exceeded that of the controls. A Ni^{++} concentration of 47.2γ per ml was inhibitory, the final population density was less than one half that of the controls at 120 hours while 59γ per ml allowed only slight growth in the same period.

Co^{++} was considerably more inhibitory, therefore, to the growth of *Tetrahymena* than equivalent concentrations of Ni^{++}. The effect of the metals appears to be mainly an inhibition of cell division as the highest concentrations employed were not lethal to the cells. The differences observed may be due to variations in permeability of the cell wall to these two ions. Since Co^{++} apparently is required by the protozoa, while Ni^{++} is probably not essential, the former ion may pass more readily through the cell membrane giving rise to higher concentrations of Co^{++} within the cell. Ni^{++} may be largely excluded even when present in high concentrations in the medium.

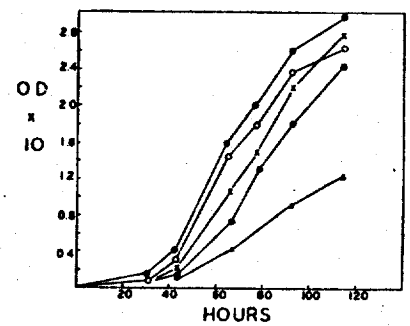

Fig. 2. The effect of Ni^{++} on the growth of *Tetrahymena pyriformis* W in peptone medium.

○——○ Controls
●——● 17.7 γ Ni^{++} per ml
×——× 29.5 γ „ „ „
◓——◓ 41.3 γ „ „ „
△——△ 47.2 γ „ „ „

The effect of Co^{++} and Ni^{++} on the RNA and DNA content of Tetrahymena.—Three 4-liter amber bottles, containing 750 ml of peptone-Marmite medium, were inoculated with 1.0 ml of 5- or 7-day-old W strain and placed on their sides. The cells were allowed to multiply at room temperature for 3 days after which Co^{++} and Ni^{++} sulfates were added, respectively, to two of the bottles to give a concentration of 29.5γ of metal ion. All three bottles were then incubated for an additional 24 hours in two experiments and 48 hours in the third experiment. At the end of the incubation period, the cells in all three bottles were counted, collected by gentle centrifugation, washed three times with distilled water and made up to a volume of 25.0 ml. The protozoa treated with Co^{++} were distinctly browner than the controls or Ni^{++} treated cells. Five ml aliquots of the concentrated cell suspension were immediately treated by the Schneider procedure and aliquots were also taken for nitrogen determinations, dry weights and metal content. The results are given in Table 1. In experiments 1 and 2 in which the protozoa were in contact with the metal salts for 24 hours, there were 17.8 and 26.1 per cent fewer cells in the Co^{++} containing media, respectively, while in experiment 1, Ni^{++} depressed the cell count by only 11.3 per cent and in experiment 2 there were 3 per cent more cells in the Ni^{++} containing medium. In keeping with this, dividing cells were noted in the controls and Ni^{++} containing media, but not in those containing Co^{++}. In spite of the decrease in cell count in the Co^{++} containing media, the amounts of N, RNA, DNA, and the dry weights of these cells were not greatly different from the controls. For the

TABLE I

The effect of Co^{++} and Ni^{++} on the nucleic acid and nitrogen content of *Tetrahymena*.

Experiment No.	Cells/ml $\times 10^{-3}$	Dry wt/ml† mg	N content/ml mg	RNA/ml mg	DNA/ml μg	Metal ion/ml μg	RNA/cell $\mu g \times 10^{2}$	DNA/cell $\mu g \times 10^{3}$
I. 24 hrs in presence of 29.5 γ/ml metal ion								
1. Control	169	17.0	1.64	2.27	348		1.34	2.06
2. Co^{++} treated .	139	16.9	1.58	2.22	356	5.55	1.60	2.57
3. Ni^{++} treated .	150	17.8	1.64	2.30	340	3.81	1.53	2.26
II. 24 hrs in presence of 29.5 γ/ml metal ion								
1. Control	194	17.6	1.68	2.55	376		1.31	1.94
2. Co^{++} treated .	120	16.6	1.66	2.31	402		1.92	3.35
3. Ni^{++} treated .	200	19.4	1.71	2.75	451		1.38	2.25
III. 48 hrs in presence of 29.5 γ/ml metal ion*								
1. Control	195	21.7	2.16	2.89	430		1.48	2.20
2. Co^{++} treated .	103	10.1	0.94	1.31	280		1.27	2.62
3. Ni^{++} treated .	174	19.8	1.96	2.79	328		1.60	1.89

* 7-day culture used for inoculation. In exp. I and II a 5-day culture was used.
† Dried at 80° for 24 hrs.

Ni^{++} treated cells the corresponding values were the same or slightly higher than the controls. As a result, the values of RNA and DNA per cell were considerably higher for the Co^{++} treated and somewhat higher for the Ni^{++} treated, than the controls. Thus Co^{++}, and to a lesser extent Ni^{++}, appear to interfere with the normal turnover, in this case presumably the breakdown by nucleases, of nucleic acids. The effect of the ions on the nuclease activity of cell free homogenates is reported in Section II of this paper.

In experiment 3, DNA per cell was also elevated but RNA per cell was less than in the controls, suggesting that other factors are beginning to operate in cells exposed to Co^{++} for 48 hours. Possibly the cells are deteriorating after this length of exposure. It is of interest to note, however, that the ratio of RNA to N is remarkably constant in all experiments in contrast to the results with bacteria [9], and it appears that protein synthesis is not greatly inhibited in *Tetrahymena* by the concentration of Co^{++} used in this experiment. In experiment 1, assay of the concentrated cell suspensions for cobalt and nickel indicated that relatively large amounts of these metals were inside or adsorbed on the surface of the cells. Present techniques do not allow us to distinguish

TABLE II

The effect of various concentrations of Co^{++} and Ni^{++} on the activity of crystalline pancreatic ribonuclease.

	Average of triplicate experiments			
	Final conc. of metal ion	Amount of enzyme per 25 ml soln.	Inhibition by Co^{++}	Inhibition by Ni^{++}
	M	γ	per cent	per cent
1	1.6×10^{-4}	2	6	9
2	4×10^{-4}	2	17	28
3	8×10^{-4}	2	31	56
4	1.6×10^{-3}	2	52	72
5	8×10^{-4}	1	52	64

the extent of these two possibilities. Approximately 30 per cent more cobalt than nickel was taken up (somewhat greater than this on a per cell basis) by the protozoa.

In Vitro Effects of Co^{++} and Ni^{++}

The action of Co^{++} and Ni^{++} on crystalline pancreatic RNase.—The effect of various concentrations of Co^{++} and Ni^{++} sulfates on pancreatic RNase is given in Table II. The experimental conditions were as follows: Ten ml of 0.2 per cent NaRNA were incubated with 1.0 ml of metal salt solution for 10 minutes and then 10 ml of 0.1M veronal-acetate buffer, pH 7.8, and 4.0 ml of enzyme solution were added. Aliquots were removed at two-minute intervals and precipitated with an equal volume of 1N HCl. The turbidity was read after three minutes in a photoelectric colorimeter. Controls utilizing 1 ml of H_2O instead of salt solution were run similarly. The salts had no effect on the precipitability of the RNA by HCl and incubation of them with the substrate for longer periods of time did not alter the results. The per cent inhibition was calculated from the ten-minute reading.

Examination of the data shows that Ni^{++} was more inhibitory than Co^{++} at all concentrations used, amounting to 72 per cent with 1.6×10^{-3} M $NiSO_4$. Decrease in enzyme concentration increased the per cent inhibition by Co^{++} markedly and by Ni^{++} slightly. Miura and Nakamura [11] reported that 6×10^{-3} M $CoCl_2$ inhibited crystalline pancreatic RNase by 12 per cent, while $NiSO_4$ at the same concentration had no effect. They used the Klein method of assay [7] but did not report the details of their experimental conditions such as substrate and enzyme concentration. It is possible that the differences between their work and the present report may be due to large differences in experimental conditions, particularly in enzyme concentration, as this appears to be an important factor. Not enough experimental data are presented in Table II to determine whether the inhibition is due to a combina-

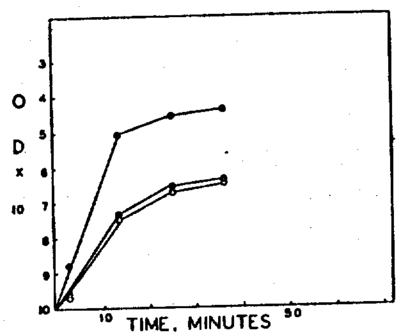

Fig. 3. The effect of Co^{++} and Ni^{++} on the activity of RNase in homogenates of *Tetrahymena pyriformis* W.
●——● Control
◓——◓ 8.0×10^{-4} *M* Ni^{++}
○——○ 8.0×10^{-4} *M* Co^{++}

tion of Co^{++} and Ni^{++} with the enzyme, substrate or both. Since the inhibition of pancreatic RNase by Mg^{++} has been attributed to the formation of a substrate-metal complex, the inhibition by Co^{++} and Ni^{++} is probably of this nature, although combination with the enzyme cannot be ruled out. Recent studies on the structure of pancreatic RNase indicate that there are no free SH groups [1] with which the metal ions might combine. As previously mentioned, the fact that Co^{++} or Ni^{++} inhibit pancreatic RNase has no bearing on the possible effects of these metal ions on other intracellular RNases which may be quite different in their properties from the pancreatic enzyme [8, 12, 15]. Studies were carried out, therefore, on the effect of Co^{++} and Ni^{++} on the RNase activity in cell-free homogenates of *Tetrahymena*.

The effect of Co^{++} and Ni^{++} on RNase activity in Tetrahymena homogenates.—Three-day cultures of *Tetrahymena pyriformis* W were grown and harvested as described above. The 25 ml concentrates were homogenized to cell-free preparations by the method described by Eichel [4] and Co^{++} and Ni^{++} added to aliquots of the homogenates to give a final concentration of 8.0×10^{-4} *M*. Assays for RNase were performed by the modified McCarty procedure [10] at pH 6.1 using veronal-acetate buffer. The results of a typical assay are plotted in Fig. 3. It may be seen that both Co^{++} and Ni^{++} were inhibitory to RNase activity to about the same degree at this concentration in distinction from the effects of the ions on pancreatic RNase where Ni^{++} was considerably more inhibitory.

DISCUSSION

When testing the effects of various substances such as metal ions on enzyme activity in crude homogenates from cells, it is difficult to assess the effects of these substances on the intact cell where the relation of the components is undisturbed, membranes are intact and active absorption or exclusion of physiologically active substances may be in progress. In addition, in some cells, the presence of two RNases and an inhibitor for at least one of them has been detected [8, 15, 16]. However, in *Tetrahymena*, no studies have been made concerning the presence of more than one RNase or an inhibitor. It is true also, that investigations of the effect of metal ions or other substances on purified enzymes and enzyme systems may be criticized on the basis that, in the cell, the components are in the presence of a complex and carefully organized milieu in which there may be many interactions. In spite of these difficulties the data presented are, at least, not inconsistent with the theory that in *Tetrahymena* the inhibitory effects of Co^{++} and Ni^{++} on cell division are due to the inhibition of intracellular RNase activity, and perhaps DNase activity, which in turn prevents the normal breakdown and turnover of nucleic acids. The fact that more Co^{++} than Ni^{++} is taken up by the cells could explain the greater activity of the former ion.

SUMMARY

The effect of Co^{++} and Ni^{++} on the growth, RNA and DNA content of *Tetrahymena pyriformis* W was determined. Co^{++} was considerably more inhibitory to growth than equivalent concentrations of Ni^{++} and the former ion resulted in a significantly increased RNA and DNA content per cell under certain conditions. The action of Co^{++} and Ni^{++} on crystalline pancreatic RNase and on RNase activity in *Tetrahymena* homogenates was also determined and the results discussed in terms of a possible function of intracellular RNase.

The author would like to express his sincere appreciation to Dr. M. Webb for his encouragement and aid in this study and to Dr. Honor B. Fell for her interest and provision of facilities for doing this work.

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Arch. Environ. Health
23:102 (1971)

Toxic Effects of Trace Elements on the Reproduction of Mice and Rats

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Breeding mice and rats were exposed to low doses of six trace elements in drinking water in an environment controlled as to contaminating trace metals. Each group was carried through three generations. Compared to control mice given only doubly deionized water, selenate (3 ppm selenium) resulted in excess deaths before weaning, runts, and failures to breed. Lead (25 ppm) and cadmium (10 ppm) resulted in loss of the strain in two generations with many abnormalities. Molybdate (10 ppm molybdenum) was slightly toxic in this respect, and arsenic resulted only in elevated ratios of males to females. In rats, lead was very toxic, and titanium and nickel moderately toxic, resulting in many early deaths and runts. This method provides fairly rapid estimates of innate toxicities of trace elements in doses tolerable for growth and survival.

TERATOGENIC effects of certain trace elements have resulted when the element was injected into pregnant animals. Thus, cadmium sulfate has produced congenital abnormalities and resorbed embryos when doses of 2.0 mg/kg of body weight were injected into hamsters on the eighth day of pregnancy.[1] Orally administered selenium compounds are teratogenic, and livestock feeding on seleniferous grasses have had dead or deformed offspring.[2] Lead has been known to be toxic in this respect for many years, and injections into hamsters have caused specific abnormalities.[3] Injected arsenic was likewise teratogenic.[4]

Submitted for publication Oct 1, 1970; accepted Dec 9.

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Innate toxic effects of 22 elements in 25 forms have been studied in this laboratory by giving small doses of soluble salts in drinking water to mice for their lifetimes (H. A. Schroeder, MD, unpublished data).[5-7] Likewise, 14 elements have been evaluated in rats.[8-10] In this way, effects on growth, survival, tumors, and longevity were ascertained. The experiments, however, were time-consuming, each lasting several years. A more sensitive and more rapid test of innate toxicity might become manifest by exposing breeding mice and rats to single elements in drinking water for several generations. Therefore, three elements with demonstrated innate toxicities, selenium, lead, and cadmium, and four elements without demonstrable long-term toxicity, arsenic, nickel, titanium, and molybdenum, were studied in mice or rats.

Methods

Mice of the Charles Rivers CD strain and rats of the Long-Evans BLU:(LE) strain were born in a laboratory especially designed and constructed to avoid environmental contamination from trace metals.[11] The diet used was composed of untreated seed rye flour (60%), dried skim milk (30%), corn oil (9%), and iodized sodium chloride (1%), with added iron and vitamins.[11] Drinking water from a forest spring was doubly deionized. To it were added single elements as soluble salts (ppm element): selenate, 3; arsenite, 5; lead, 25; molybdate, 10; cadmium, 10; nickel, 5; and titanate, 5.

The diet contained the following elements (ppm wet weight): zinc, 23.2; copper, 1.95; manganese, 12.25; cobalt, 0.18; molybdenum, 0.45; selenium, 0.056; and chromium, 0.16; as well as the abnormal elements, arsenic, 0.06; lead, 0.20; cadmium, 0.10; nickel, 0.31; and titanium, 1.12.

Five pairs of mice or rats were randomly selected for each element from divided litters at the time of weaning, placed in separate cages and given the element in drinking water continuously. Mice were allowed to breed as often as they would up to 6 months of age and rats up to 9 months of age or longer. At weaning time, pairs were randomly selected from the first litter (F_1A generation), from the second litter (F_1B), and sometimes from the third (F_1C) and allowed to breed as they would to produce the F_2 generations. Surplus surviving animals were discarded at the time of weaning, after ascertaining the sex.

Pairs were likewise selected at random from the first F_2A litters and allowed to breed the F_3A generation, and sometimes from the second litter for the F_2B generation. Experiments were terminated when the strain was obviously dying out or when three generations had been weaned. Control animals received plain deionized water and were treated similarly.

Criteria used to estimate innate toxicity were the intervals between litters, the age at which the pair produced its first litter, the ratio of males to females, and the numbers of runts (animals with large heads and small bodies), deaths, stillborn offspring, failures to breed, and congenital abnormalities.

The doses of trace elements in drinking water were those which we have found, by lifeterm experiments, not to affect the rates of growth of mice and rats as compared to controls, not to affect reasonably long-term survival or cause early mortality, and, except in the case of lead, not to affect longevity. In these doses, arsenic, nickel, molybdenum, and titanium showed no innate toxicity; cadmium induced arterial hypertension; lead shortened life span; and selenate produced malignant tumors (H. A. Schroeder, MD, unpublished data).[5-12]

Results

Effects of Selenate, Arsenite, Lead, Molybdate, and Cadmium in Mice.—Control mice bred normally for four generations, with average litters of 10 to 11 offspring (Table 1). There were no stillbirths, one maternal death, seven deaths of young before weaning, two runts, and no congenital abnormalities. There were no signs of deterioration as this strain became inbred. The average age of a pair at birth of the first litter was 64 days, and for F_2 and F_3 litters, 62 to 64 days. The average intervals between litters were 28 to 30 days, and the ratios of males to females were 0.94 to 1.03.

In the cases of mice given lead, the strain died out rapidly, so that by the second generation there were insufficient numbers to continue (Table 2). Only eight litters, two of them stillborn, appeared in the F_1 generation and only two in the F_2. There were nine deaths of young mice and 69 runts out of 72 live offspring (95.6%) in the F_1 generation, of which four grew enough to breed at 70 to 75 days of age.

Selenium was toxic according to these criteria. The strain did not begin to die out until the F_3 generation, which produced only three litters with 23 animals, of which 16 were runts. Seven pairs failed to breed. In all, there were 23 young deaths, one stillborn litter, 93 runts out of 389 live offspring (23.9%) and one maternal death. The male-female ratios ranged from 1.27 to 1.65 in the various subgroups.

Cadmium was also toxic to breeding mice, with the strain not lasting beyond the second (F_2B) generation. A congenital abnormality, sharp angulation of the distal third of the tail, appeared in five litters, comprising 4 of 255 live offspring (16.1%) of the F_1 and F_2A generations. There were also 34 runts (13.3%) in the offspring living beyond weaning, 87 deaths before weaning (30.5%), two maternal deaths, and two dead litters. Three of five pairs failed to breed in the F_2B generation, and the experiment was discontinued.

Molybdenum in the dose used was moderately toxic, in that there were 15 early deaths of 238 offspring in the F_1 generation (6.3%), seven early deaths of 242 offspring in the F_2 generation, and five dead litters with one maternal death in the F_2 generation. The F_3A generation of four pairs died out, as two failed to breed and there were only two litters, one of 11 runts and one of two males. The F_3B generation had one failure to breed, one dead litter and mother, 67 offspring of which 13 died, and a litter with only one male. The F_3C generation had 21 deaths of 43 offspring, three dead litters, and three maternal deaths. The experiment was discontinued.

Table 1.—*Summary of Breeding Experiments in Mice Exposed to Trace Elements*

	Control	Selenium	Arsenic	Lead	Molybdenum	Cadmium
F_1 generation						
No. of litters	19	16	19	8	21	14
Pair age at first litter, days	64	67	70	70.3	64.4	80.0
Interval between litters, days	30	34	28	48	30	34.6
Average litter size	11.0	12.3	8.2	12.0	11.3	13.1
M-F ratio	0.94	1.50	0.93	1.05	1.05	1.05
F_2 generation						
No. of litters	23	17	25	2	26	11
Pair age at first litter, days	64	54	69	73	66	69
Interval between litters, days	28	33	32	...	43	56
Average litter size	10.3	10.0	9.6	11.5	10.3	9.2
M-F ratio	1.03	1.44	1.30	0.92	1.32	0.78
F_3 generation				Discontinued		Discontinued
No. of litters	22	3	7		14	
Pair age at first litter, days	62	54	63		79	
Interval between litters, days	28	...	22		25	
Average litter size	10.5	7.6	8.1		8.8	
M-F ratio	1.00	1.30	1.71		0.90	

Table 2.—*Deaths and Abnormalities of Mice Bred While Exposed to Trace Elements*

	Control	Selenium	Arsenic	Lead	Molybdenum	Cadmium
F_1 generation						
Maternal deaths	0	0	0	0	0	0
Dead litters	0	1	0	2	0	0
Young deaths	0	13*	1	9†	15‡	39†
Failures to breed	0	2	0	0	0	0
Runts	0	36*	0	69†	0	25†
No. of mice	209	197	147	72	238	184
F_2 generation						
Maternal deaths	1	1	0	0	1	2
Dead litters	0	0	0	0	5	2
Young deaths	6	10	7	0	7	48†
Failures to breed	0	2	1	1	1	3
Runts	2	41†	0	0	2	9*
No. of mice	248	169	290	23	242	101
F_3 generation				Discontinued		Discontinued
Maternal deaths	0	0	0		4§	
Dead litters	0	0	0		4§	
Young deaths	1	0	0		34†	
Failures to breed	0	3	0		3	
Runts	0	16†	0		11†	
No. of mice	230	23	57		123	
Total No. of mice	687	389	494	95	603	285

* Differs from controls by χ^2 analysis; $P < 0.0005$.
† $P < 0.0001$.
‡ $P < 0.001$.
§ $P < 0.05$.

The mice fed arsenic survived well through the third generation. There were no runts in 494 progeny, eight young deaths, one failure to breed, and no maternal deaths. The ratio of males to females increased from 0.93 to 1.71 (Table 1). The only other abnormality noticed was a reduction in size of the litters, with the mean size in each successive generation being 8.2, 9.6, and 8.1 mice. In fact, eight of 51 litters (16.0%) had 2 to 5 mice, compared to four of 61 in the molybdenum group (7.4%), one of 64 in the controls (1.6%), 3 of 36 in the selenium group (8.3%), none of eight in the lead group, and none of 25 in the cadmium group. The number of small litters in mice

Table 3.—*Summary of Breeding Experiments in Rats Exposed to Nickel, Titanium, and Lead*

	No. of Litters	Pair Age at First Litter, Days	Interval Between Litters, Days	Average Litter Size	M-F Ratio
F_1 generation					
Control	10	89	41	11.4	1.14
Nickel	11	101	42	11.0	1.20
Titanium	11	87	49	9.4	1.43
Lead	19	139	43	9.1	0.68
F_2 generation					
Control	10	87	40	11.3	1.10
Nickel	15	98	38	10.5	1.18
Titanium	16	87	39	10.9	0.99
Lead	32	92	38	10.3	1.16
F_3 generation					
Control	11	86	41	11.0	1.06
Nickel	10	92	30	8.1	0.44
Titanium	2	88	...	8.0	0.60
Lead	6	93	30	8.0	0.91

fed arsenic differed from that in the controls ($P < 0.05$).

Effects of Lead, Nickel, and Titanium on Breeding of Rats.—Lead was toxic to breeding rats, but to lesser degree than it was to mice (Table 3 and 4). Birth of the first litters was somewhat delayed. In the first generation there were 12 deaths before weaning, two maternal deaths, 40 runts, and a male-female ratio of 0.68, in 19 litters of 173 offspring. The five pairs of the F_2A generation produced 12 litters of 133 rats, of which 20 died and five were runts. In the F_2B generation there were 13 litters of 132 rats, with ten runts and seven deaths. Four of their five matings produced two F_3B litters of 22, with two deaths, one dead litter, and one failure to breed. This line was, therefore, discontinued. The F_2C generation had four of five successful matings, with seven litters of 46 rats, one dead litter, eight deaths, and 11 runts. Three litters had only three to four rats each. Two pairs of F_3C rats failed to reproduce, and this experiment was discontinued.

Nickel was less toxic than lead in respect to breeding, with 9.1% young deaths, one maternal death, and 30.6% runts in the first generation; 10.2% young deaths and 5.1% runts in the second; and 21.0% (17) young deaths and 6.2% (5) runts in the third. The size of the litters decreased somewhat with each generation, and, with two failures to breed, the number of rats was reduced. Few males were born in the third generation.

Titanium was nearly as toxic as nickel,

Table 4.—*Deaths and Abnormalities in Rats Bred While Exposed to Nickel, Titanium, and Lead*

	Control	Nickel	Titanium	Lead
F_1 generation				
Maternal deaths	0	1	0	2
Dead litters	0	0	0	2
Young deaths	0	11*	1	12†
Failures to breed	0	0	0	1
Runts	0	37‡	23‡	40‡
No. of rats	114	121	103	173
F_2 generation				
Maternal deaths	0	0	1	0
Dead litters	0	0	0	1
Young deaths	0	16‡	25‡	35‡
Failures to breed	0	2	4	3
Runts	1	8	14†	26§
No. of rats	113	157	174	311
F_3 generation				
Maternal deaths	0	0	0	0
Dead litters	0	0	0	1
Young deaths	1	17‡	0	2
Failures to breed	0	0	0	1
Runts	0	5†	6‡	4§
No. of rats	121	81	16	22
Total No. of rats	348	359	293	506

* Differs from controls by χ^2 analysis; $P < 0.005$.
† $P < 0.0001$.
‡ $P < 0.025$.
§ $P < 0.01$.

with a marked reduction in the numbers of animals surviving to the third generation. In the three generations there were 8.9% young deaths, 16.4% runts in the offspring living beyond weaning, and four failures to breed. Only two litters appeared in the third generation. The male-female ratio was progressively reduced. The controls continued to breed for four generations, with rare deaths and runts occurring.

Comment

These experiments provide a much more sensitive method for detecting toxicity of an element than feeding the element for life. Thus, selenate increased the incidence of spontaneous tumors and malignant tumors in mice and rats, when fed for a lifetime, without altering life span (H. A. Schroeder, MD, unpublished data). Lead shortened life span at this dose (25 ppm) without interfering with growth[5,8]; the effect on breeding mice and rats was unexpected. Cadmium feeding to rats resulted in arterial hypertension.[12] Injection of cadmium resulted in toxemia of pregnancy,[13] and, in males, testicular atrophy has been induced.[14] Toxic effects of cadmium can be antagonized by zinc or selenium.[1] Partial breeding out of the strain given molybdenum was unexpected, for no adverse metabolic effects have resulted from this dose fed to rats for life. The minor effect of arsenic was consistent with the absence of effects in rats and mice fed this dose for life.[6,9] Nickel fed for life was not toxic to rats (H. A. Schroeder, MD, unpublished data) or to mice,[5] nor was titanium toxic to mice.[5]

Therefore, feeding of these trace elements resulted in relative toxicities in the following order: lead > cadmium > selenium > nickel, arsenic, titanium, molybdenum. Exposure of breeding mice and rats resulted in the following relative toxicities: lead = cadmium > selenium > nickel > titanium > molybdenum > arsenic.

Developing males in utero are believed to be more vulnerable to toxic substances than are developing females. In these experiments, however, the ratio of males to females born was increased in mice exposed to selenium and arsenic, compared to controls. This ratio became reduced in mice exposed to cadmium and in rats exposed to titanium and nickel. The mechanisms of these alterations are unexplained.

From these experiments it is clear that certain trace elements fed to rats and mice in doses which do not interfere with growth or survival are intolerable for normal reproduction.

This investigation was supported by Public Health Service grant-in-aid 05076 and by grants from CIBA Pharmaceutical Products, Inc., Cooper Laboratories, Inc., and General Foods Corp.

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ABNORMAL TRACE METALS IN MAN—NICKEL*

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(*Received* 14 *April* 1961)

NICKEL is one of the relatively nontoxic trace metals found in the tissues of man, ranking in this respect with the essential elements, iron, cobalt, copper and zinc. Its physiological role, if any, has not been established and there have been few biological studies of this transitional metal [1]. A great deal is known, however, about its chelates and complexes [2]. Nickel has been found in soils, in a variety of plants, in sea foods and in many organs and tissues of animals [1]. It may also enter foods during processing [3]. Few analyses on the sources of nickel in man have been made and little is known of this interesting but relatively neglected element. The present report is concerned with a re-examination of nickel, its possible physiological role and its presence in human beings around the earth. Our purpose is to attempt to answer three questions: (1) What are the sources of the nickel found in man? (2) Is nickel of natural occurrence in food or does much of it come from industrial contamination? (3) Is nickel an essential trace metal in the sense that it has a definite physiological function?

METHOD

Samples were ashed at 450°C in a muffle furnace after drying to constant weight. Water was evaporated to 5 or 6 ml, treated with 1 ml concentrated HNO_3 and evaporated to dryness. The residues were dissolved in de-ionized water having a resistance of one million ohms or more. Nickel was measured by the microanalytical method of Sandell for biomaterial, which depends upon formation of a color with dimethylglyoxime. Optical density was read at 465 mμ in a Bausch and Lomb Spectronic 20 Colorimeter or a Beckman DB spectrophotometer. Recoveries of known amounts of nickel to unknowns were usually 100 per cent. Limits of sensitivity were about 0·05 μg per sample. No nickel was detected in the reagents used. Ammonia was triply distilled. The raw data of TIPTON *et al.* [4, 5–9] on the trace metal concentrations of human tissues from the United States and foreign countries‡ were obtained by spectrographic methods.

CONCENTRATION OF NICKEL IN HUMAN TISSUES

In Table 1 are shown the mean concentrations and the per cent occurrence of nickel in adult human kidneys and livers from various areas of the world [4]. From these data it would appear that nickel is more prevalent in these two organs in some foreign persons than it is in most Americans. This difference did not hold for lung.

*With the technical assistance of Frances S. Gibson, A.B. and Shalini N. Valanju, M.Sc.

†Under grant-in-aid H-5076 from the National Heart Institute, U.S. Public Health Service, Ciba Pharmaceutical Products and the Vermont Heart Association.

‡The African tissues were collected by Dr. H. M. Perry, Jr., the other foreign tissues by one of us (H.A.S.) and the American by Miss M. J. Cook.

TABLE 1. CONCENTRATION OF NICKEL IN KIDNEY AND LIVER BY GEOGRAPHICAL LOCATION (Means, p.p.m. ash).

Area	Kidney No.	Kidney Mean	%*	Liver No.	Liver Mean	%*
United States	161	7	27	163	6	22
Alaska	2	35	100	1	36	100
Honolulu	5	4	40	5	4	40
Bern	9	11	100	9	7	67
Tokyo	10	7	80	10	10	70
Kyoto	11	10	55	9	8	67
Taiwan	9	16	78	9	10	89
Hong Kong	10	9	60	10	5	20
Manila	4	12	75	4	45	75
Bangkok	10	7	50	10	7	30
Bombay	9	28	89	9	20	56
Vellore	11	25	9	13	0	0
Delhi	10	22	100	10	14	90
Beirut	6	0	0	6	0	0
Cairo	3	5	33	2	0	0
Nigeria	19	8	58	17	30	6
Lambarene	5	<5	20	5	0	0
Welkom	5	23	80	3	0	0
Uganda	4	0	0	4	0	0
Usumbura	11	12	64	11	9	82
Totals, excluding U.S.	146	12·4	58·2	141	11·0	44·0

*% Occurrence

The median per cent ash of kidneys was 1·1 (90 per cent range 0·8–1·3) and of livers 1·3 (90 per cent range 1·0–1·8).

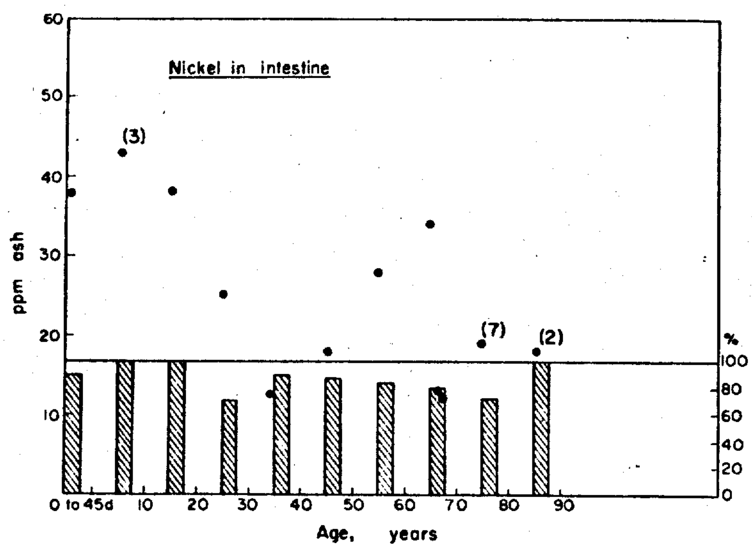

FIG. 1. Concentration of nickel in intestine according to age (137 samples), United States only. Black dots, mean concentrations; hatched bars, frequencies of occurrence. Note presence of nickel in newborn.

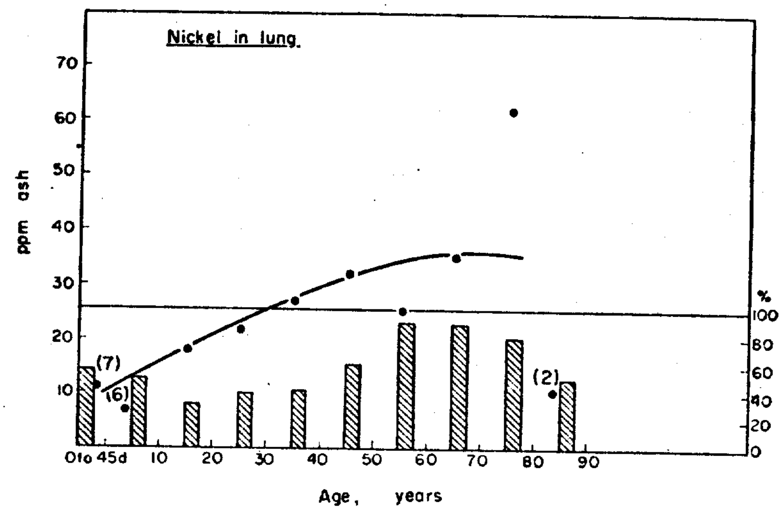

FIG. 2. Concentration of nickel in lung according to age (156 samples), United States only. Black dots, mean concentrations; hatched bars, frequencies of occurrence. Note presence of nickel in newborn.

The raw spectrographic data on American human tissues were examined by age and the published data [5–9] by city of origin. The relative infrequency of the appearance of detectable nickel in most tissues made statistical analyses unrewarding. Its occurrence in 1,154 samples of eleven tissues was as follows: Bone 5 per cent; liver 25 per cent; larynx 31 per cent; kidney 38 per cent; heart 42 per cent; trachea 49 per cent; aorta 49 per cent; lung 56 per cent. On the other hand, it was found in 87 per cent of intestines and skins. Fig. 1 shows the frequency and mean concentrations of nickel in intestines according to age. There is no consistent trend. In the other tissues also, except lung, there were no significant increases either in frequency of occurrence or concentration with age. In the case of lung, however, frequency increased from about 30 to 40 per cent in the first four decades to 90 per cent in the sixth and seventh, while the concentration likewise increased (Fig. 2). Nickel was present in lung, liver, kidney and intestine of most stillborn infants.

TABLE 2. NICKEL IN AMERICAN TISSUES BY AREA (From TIPTON *et al.* [5–9]).

City of origin	Date†	No. samples*	No. positive	%	No. trace	Total %	Kidney and liver %
Miami, Florida	2/28/57	387	18	4·6	2	5·2	0·0
Denver	3/12/56	268	27	10·1	2	10·8	4·2
Dallas	2/28/57	370	38	10·2	9	12·7	5·5
Baltimore	11/11/57	414	87	21·0	17	25·1	59·2
Richmond, Va.	8/25/59	391	64	16·7	45	28·0	26·4
Seattle–Tacoma	10/6/58	296	94	31·7	10	35–2	26·9
New York–Chicago	12/10/54	233	172	74·0	3	75·0	69·0

Limit of sensitivity of method, 5 p.p.m. ash

*Excluding intestine
†Reported

TABLE 3. NICKEL IN FOOD

Sample	µg per g (wet weight)	µg per 100 g	µg per 100 calories*
Condiments			
Salt, table	0·35	—	—
Pepper, black	3·93	—	—
Baking powder	13·40	—	—
Sugar, cane	0·03	3	1
Yeast, dry active	0·48	—	—
Cinnamon	0·74	—	—
Nutmeg	1·17	—	—
Allspice	0·79	—	—
Bay leaves	0·88	—	—
Cloves, whole	0·10	—	—
Sea food			
Oysters, fresh	1·50	150	300
Clams, fresh	0·58	58	121
Scallops, fresh frozen	0·04	4	4
Lobster, claw meat	0·66	66	55
Shrimp, fresh frozen	0·03	3	3
Crabmeat, canned	0·03	3	2
Anchovies, canned	0·72	72	36
Sardines, canned	0·21	21	7
Kippered herring, canned	1·70	170	85
Haddock, frozen	0·05	5	7
Swordfish, frozen	0·02	2	2
Meats			
Lamb chop	0·0	—	—
Pork chop	0·02	2	1
Pork chop	0·0	—	—
Beef, chuck	0·0	—	—
Beef, round	0·0	—	—
Beef, marrow	0·22	22	6
Gelatin	4·50	450	173
Egg, whole	0·03	3	2
Grains			
Wheat, winter, seed	0·16	16	5
Wheat, Japanese	0·0	—	—
Wheat, Japanese	0·0	—	—
Wheat flour, Japanese	0·0	—	—
Wheat flour, all-purpose	0·54	54	15
Wheat flour, all-purpose	0·30	30	8
Wheat, crushed, Vermont	0·75	75	21
Bread, whole-wheat, stone-ground	1·33	133	53
Wheatena	0·0	—	—
Wheaties	3·00	300	75
All-bran cereal	0·74	74	25
Grapenuts cereal	0·13	13	4
Buckwheat, seed	6·45	645	194
Rye, seed	2·70	270	80
Oats, seed	2·60	260	65
Oats, seed	1·71	171	43
Oats, precooked, quick	2·35	235	59

*Calorie values from MCCANCE, R. A. and WIDDOWSON, E. M.: *The Chemical Composition of Foods*. Chemical Publishing, Brooklyn, 1947.

TABLE 3—*continued*

Sample	μg per g (wet weight)	μg per 100 g	μg per 100 calories*
Grains—continued			
Corn, frozen, fresh	0·70	70	20
Corn meal, New Hampshire	0·0	—	—
Corn oil	0·0	—	—
Rice, Japanese, polished	0·50	50	14
Rice, Japanese, unpolished	1·80	180	50
Rice Japanese, polished (204) samples	0·65	65	19
Rice, American, polished	0·47	47	131
Rice, puffed	0·30	30	10
Vegetable shortening, hydrogenated	1·14	114	13
Vegetables			
Potato, raw	0·56	56	61
Peas, fresh frozen	0·30	30	47
Peas, canned	0·46	46	54
Peas, split, dried	1·66	166	55
Beans, string, frozen	0·65	65	930
Beans, string, canned	0·17	17	189
Beans, Navy, dried	1·59	159	52
Beans, yellow-eye, dried	0·69	69	23
Beans, red kidney, dried	2·59	259	100
Spinach, fresh	0·35	35	135
Celery, fresh	0·37	37	411
Beet greens	1·94	194	1763
Swiss chard, organic	0·71	71	260
Escarole, fresh	0·27	27	245
Chicory, fresh	0·55	55	611
Lettuce, garden, organic	1·14	114	1364
Lettuce, head	0·14	14	127
Kale, organic	1·12	112	728
Kohlrabi, leaves, organic	0·47	47	235
Cabbage, white	0·32	32	160
Cabbage, white	0·14	14	70
Cabbage, red	0·24	24	120
Cauliflower leaves	0·19	19	173
Broccoli, fresh, frozen	0·33	33	235
Tomato, fresh	0·03	3	2
Tomato juice, canned	0·05	5	4
Apple, raw	0·0	—	—
Apple, raw	0·08	8	18
Banana	0·34	34	50
Pear	0·20	20	50
Fluids			
Milk, whole, fresh	0·0	—	—
Milk, evaporated	0·03	3	2
Milk, evaporated	0·03	3	2
Milk, dry, skim packaged	0·0	—	—
Milk, dry, skim, bulk	0·0	—	—
Tea, Orange Pekoe	7·60	—	—
Cocoa	5·00	500	111
Cola	0·0	—	—
Ginger ale	5/l	—	—
Cider	550/l	55	122
Cider vinegar	315/l	32	—

TABLE 3—*continued*

Fluids—continued			
Beer, canned	10/l	1	2
Mineral water, bottled, Arkansas	12·5/l	1	—
Cigarettes, whole, filtered	0·0	—	—
Animal food			
Poultry wheat	0·41	41	12
Dog food, commercial	2·09	209	51
Rat diet, commercial	3·33	333	88
Rat food (rye, milk, corn oil)	0·20–0·68	20–68	5–17

There was some geographical distribution in the United States for the frequency of nickel's presence in one or more tissues, excluding intestine. It was increased in samples from New York–Chicago, and Seattle–Tacoma, as compared to those from Miami, Denver and Dallas [5–9] (Table 2).

NICKEL IN FOOD AND WATER

In Table 3 are the nickel concentrations in a variety of foods and beverages according to the microanalytical chemical method. A few foods may have obtained nickel during processing (two breakfast cereals, apple cider, animal foods, gelatin, baking powder), but in most it apparently occurred naturally. The relative absence of this metal in most animal products is noteworthy.

No nickel was found in a number of fresh tap waters analyzed. It appeared, however, in concentrations of 6·5 to 9·5 p.p.b. in a newly installed water system from a forest spring, consisting of 210 ft. of black polyethylene pipe, 10 ft. of copper, and a "glass-lined" gas water heater. After a month it had disappeared. There was none detected at the source. One other rural spring with lead pipe showed 12·5 p.p.b. at the tap. No nickel was detected in eight samples of snow from various country and suburban locations, in some of which lead and cadmium were found [10]. One sample directly contaminated by automobile exhaust fumes contained 0·56 p.p.b.

In order to detect solution of nickel from metal, stainless steel animal drinking tubes were left in a soft (18 p.p.m. hardness), acid (pH 6·0), spring water for 2 weeks. The water dissolved nickel to a concentration of 11 μg per liter.

NICKEL IN ANIMAL TISSUES

The amounts of nickel in various tissues of domestic and wild animals are shown in Table 4. The absence of this metal in red squirrel and most deer is unexplained. In an attempt to discover sources, wild vegetation eaten by animals and birds was analyzed (Table 5). Nickel appeared constantly in the natural substances, except for old wood from a large elm tree, where it was concentrated in the white wood and bark.

NICKEL IN THE LOCAL ENVIRONMENT

A variety of building and other materials were examined for the presence of nickel (Table 6). It appeared to be relatively ubiquitous, especially in products made from wood, including common substances used for animal bedding. As stainless steel also contains nickel, the difficulty of obtaining a nickel-free environment for animals is clear, even if the diet contained none.

TABLE 4. NICKEL IN ANIMAL TISSUES

Sample	µg/g (wet weight)	
Kidney		
Robin	1·66	
Ruffed grouse	4·96	
Red squirrel, male	0·0	
Red squirrel, female	0·0	
Gray squirrel, male	3·19	
Rabbit, wild	0·0	
Rabbit, wild	0·08	
Deer	0·0	
Deer	2·9	
Deer	0·0	
Deer	0·0	
Deer	0·0	
Deer	0·0	
Deer	0·0	
Deer	1·73	
Deer	0·66	Spectrographic
Deer	0·52	Spectrographic
Deer	<0·16	Spectrographic
Deer	0·0	Spectrographic
Beef	0·66	
Pork	1·0	
Pork	3·4	
Pork	0·0	
Human, mean	<0·09	[12]
Liver		
Salmon, 1 year old	0·28	
Salmon, 4+years old	0·14	
Robin	0·91	
Ruffed grouse	2·42	
Ruffed grouse	1·11	
Red squirrel, female	0·0	
Red squirrel, male	0·0	
Gray squirrel, male	1·51	
Rabbit, wild	2·33	
Deer (§3)	2·5	
Deer (§2)	0·0	
Deer (§4)	0·0	
Deer (§5)	0·0	
Human, mean	<0·11	[12]
Heart		
Gray squirrel	3·67	
Human, mean	<0·12	[12]
Bone		
Beef	0·58	
Muscle		
Lamb	0·0	
Pork	0·0	
Beef	0·0	
Human, mean	<0·11	[12]
Aorta		
Human, aged 82	0·9	
Human, mean	<0·15	[12]

TABLE 5. NICKEL IN VEGETATION

Sample	µg/g (wet weight)
Apple leaves, wild (§1)	1·96
Apple twigs (§1)	1·87
Apple bark (§1)	5·92
Apple leaves, wild (§2)	0·76
Apple twigs (§2)	0·24
Apple (§2)	0·47
Apple leaves, wild (§3)	3·01
Apple twigs (§3)	1·40
Apple (§3)	0·31
Hemlock needles, wild	0·97
twigs	0·03
bark	0·00
Spruce needles, wild	1·69
twigs	1·31
Pine needles, wild	2·44
twigs	0·92
Elm, section 1865–1870, urban	0·10
1900–1910	0·00
1940–1947	0·00
1956–1960	1·15
bark and cambium	0·56
Sawdust, pine	0·65
Wood shavings, soft wood	0·32
Sumac berries	1·10
Peat moss, sphagnum	0·62
Sea weed, floating	0·75
Cow manure, fresh	0·75
Phosphate, rock, Tennessee	33·00
Fertilizer, 0-10-5	14·60
Fertilizer, 0-20-0 phosphate	5·38

TABLE 6. NICKEL IN MISCELLANEOUS MATERIAL

Sample	µg/g (wet weight)
Paper towel	1·43
Paper, excelsior, white	2·07
Paper, newsprint, new	0·64
Paper, building	0·06
Miracle wood	0·40
Plastic wood	0·08
Cellophane excelsior, white	19·50
Polyurethane foam	3·42
Scotch tape, black	6·67
Scotch tape, glass fibre	12·22
Cork, rubber (A. H. Thomas)	1·88
Shellac	0·05
Varnish	0·18

DISCUSSION

This report is the third of a series on the sources and geographical distributions of certain "abnormal" trace metals present in the tissues of man. By "abnormal" is meant those metals for which no function is presently known. The history of trace metallurgy is short, however, essential metals having been discovered at the rate of about two per decade, so that some of those we are considering may prove to have physiological roles.

Of the twenty-nine interesting trace elements found in the tissues of man, nickel ranks in abundance as follows: In the universe third, in the solar atmosphere third, in the earth's crust eleventh, in sea water fifteenth [11], and in the body of man fifteenth [12]. Coal contains about the same amount as lucerne grass, where it ranks twelfth [13]. There are approximately 10 mg in the "Standard Man", with wide individual variations [12]. Therefore, nickel is apparently not concentrated in man against an environmental gradient, as is lead [10] and cadmium [14].

According to GOLDSCHMIDT, the concentration of nickel in the upper lithosphere is 100 p.p.m. [15]. Its ionic radius is close to that of cobalt and iron. Much, but not all, of the nickel in soil, especially alkaline soil, is insoluble; fertility is reduced by soluble nickel and acidification can release it to the point of toxicity to citrus trees and other vegetation.

On viewing the raw data [4], one is struck by the relatively large amounts of nickel appearing in the tissues of one individual while none was detectable in the tissues of another (except for skin and intestine). Apparently there was some geographical influence operating. Kidney, liver and lung were chosen as tissues possibly influenced by environmental nickel. Of these three, it was found in two of seventy-eight samples from Miami, ten of seventy-two from Denver, and eight of eighty-three from Dallas, while twenty-six of sixty from Seattle–Tacoma, thirty-three of seventy-eight from Richmond and fifty-six of seventy-one from New York–Chicago contained this element [5]. From Table 1 a similar bizarre distribution is evident from much smaller numbers of cases: little or none in kidney and liver from Uganda, Lambarene, Vellore, Cairo and Beirut, while relatively frequent in the same tissues from Tokyo, Taiwan, Manila, Bombay, Delhi and Usumbura. No evident pattern appears which explains these differences.

Nickel in food

One tendency for the concentration of nickel in foods is seen in Table 3. Most whole grains appeared to have considerable amounts, the largest quantities being in buckwheat, rye, oats, corn, rice and one wheat. There was less in milled products, suggesting concentration in the germ. Japanese wheat and flour were exceptions. The fairly large amounts in legumes, tea and cocoa were also noteworthy. We did not find an American vegetable without nickel; two of four fruits were relatively deficient. On the other hand, the low values in piscine and mammalian muscle, eggs and milk are striking.

Calculations of a 2,300 caloric diet of 100 g protein, 250 g carbohydrate and 100 g fat would provide the following amounts of nickel: (1) Using meat, milk, eggs, fruit, refined white bread, wheatena, butter and corn oil, three to ten μg. (2) Using oysters, meat, milk, eggs, oats, whole wheat or rye bread, certain vegetables, potatoes and legumes, with little added fat, 700 to 900 μg. The average daily intake can be estimated at roughly 300 to 600 μg; calculations for four laboratory workers came to 305, 340, 360 and 480 μg. An institutional diet with beverages of about 2,040 calories weighing 2,526 g contained 472 μg of nickel, of which 245 μg were in the 550 caloric breakfast (including oatmeal, griddle cakes and toast), 137 μg in the 800 caloric dinner and 90 μg in the 690 calorie supper (which included macaroni and cheese, and fruit salad). This intake is larger than the calculated daily exposure to lead (290 μg) [10] and to cadmium (23 μg) [14], both of which accumulate in man's tissues. Obviously, if nickel is an essential trace element, certain diets (such as No. 1) could be considered as deficient, while more balanced rations (such as No. 2) would contain adequate nickel and a strictly vegetarian regimen would have much.

Nickel was found in river water seventeen times out of fifty-one locations on seventeen major rivers of the United States, in a survey conducted by the U.S. Public Health Service [16], in amounts ranging from 1 to 30 μg per liter. It was not detected by the spectrographic methods used in waters of the Mississippi, Missouri, Red, Savannah, Snake and Yellowstone Rivers.

Nickel was widely distributed in the air from all American cities studied by TABOR and WARREN [17], significant quantities being detected in 90·7 per cent of samples. In 65 per cent there was less than 0·02 μg per m³, in the remainder up to 0·2 μg. The extreme ranges were 0·005 μg per cubic meter in suburban Houston and 0·2 μg per cubic meter in East St. Louis and New York. The absence in local snow suggests that urban nickel comes from industrial contaminants and a small amount from motor vehicle exhausts. At a respiratory volume of 15 m³ per day, the amount inspired in cities would vary from 0·075 to 3·0 μg, in two-thirds of the locations being less than 0·3 μg, provided that exposures were continuous. This quantity represents a very small increment of the total daily intake, although it could account for the accumulation of the 0·4 mg in lung if deposited in insoluble form at the rate of 10 μg per year.

The curious distribution of nickel in the kidneys and livers of wild animals is unexplained, varying from none to large amounts. Sources are most likely buds, twigs, berries and leaves of trees and bushes. In forest vegetation nickel is probably of natural occurrence, for there is no reason to believe that contamination from industrial sources is appreciable in these samples.* GOLDSCHMIDT has detected it in most forest litters [15]. The presence of much nickel in phosphate fertilizers suggests that this source might influence contents of garden products.† Divalent nickel forms a stable chelate with phosphate and has been found in natural deposits [15].

In spite of the relatively large amounts in food, there appears to be a mechanism in mammals limiting intestinal absorption, just as there is for iron, manganese, copper and probably other essential metals. Large doses are required to overcome this mechanism. PHAHTAK and PATWARDHAN [18] fed rats large amounts (250 to 1,000 p.p.m.) of nickel as the carbonate for two months without evident toxicity. Bone accumulated the major portion (135 to 358 p.p.m.) with 10 to 50 p.p.m. appearing in other tissues. The proportions excreted in urine (1·56 per cent) and feces (74·4 per cent) were little affected by the levels of intakes. Nickel has been found in bile, a presumed mechanism for fecal excretion [1].

The amounts in normal human urine are said to be small or negligible [3]. PERRY and PERRY [19] found, by spectrographic methods, a daily excretion of 28 μg (±S. E. 2·59) of

*WARREN [39] has reported an average content of 20 p.p.m. ash in hemlock twigs, with up to 340 p.p.m. in wild hemlock growing near deposits of nickel ores. High metal contents of twigs and leaves of deep-rooted trees are used to detect ore-bearing rock in the area.

†From 1954 to 1959 world production of phosphate rock increased about 38 per cent, United States production 11 per cent and Florida production 7 per cent. About 90 per cent of the last was used for agriculture. Japan used about 40 per cent of total exports [36]. If our analyses are correct, fertilized soils in the United States gained about 194 long tons of nickel and 38 long tons of cadmium [14] from this source in 1959.

nickel by twenty-four normal persons in St. Louis. The amounts were quite constant and were uninfluenced by acidity, alkalinity, restricting or forcing of fluids. If we accept 28 μg as the average urinary excretion and 0·5 mg as the approximate daily intake, it is apparent that about 5 per cent of the amount ingested is absorbed. There was little tendency found for nickel to accumulate in tissues, except in lung. Its virtual absence in bone (five of 110 cases) suggests that very large intakes are required to deposit it there.

The nickel in human tissues is probably chelated quite strongly, for ten daily intravenous injections of ethylenediaminetetraacetate did not change the excretion [19]. This chelating agent has a stronger affinity for nickel than for any other metal of the first transitional group.

The toxicity for mammals is low, ranking with the essential metals and with chromium, tin, barium and silver [20]. The total bodily content is about 1 per cent of the toxic dose, if that, for nickel salts exert their action mainly by gastro-intestinal irritation and not by inherent toxicity [1, 21]. For more primitive forms of life, however, toxicity is slightly greater; for *Daphna magna*, nickel ranks higher than tin, barium, manganese, cobalt and trivalent chromium. It has a low toxicity for sticklebacks, goldfish and the flatworm, *Polycelis nigra* [20].

In nickel refineries, respiratory tract neoplasia is fairly frequent among exposed workers and nickel dermatitis is common. Nickel carbonyl has been implicated; most metal-carbonyls are toxic, including that of iron. Toxicity of a carbonyl does not indicate toxicity of the metal ion.

The answer to the question on the possible contamination of foods by industrial nickel is quite evident. From our data, nickel is ubiquitous in plants. A few prepared foods had unusually high values which undoubtedly represent contamination by processing, probably from nickel-plated vessels. For the most part, however, there was little evidence of contamination by canning or processing. Air appears to contain very little nickel. Although we found none in the ash of one brand of cigarettes, others have reported 1·9 p.p.m. in tobacco and 2 μg per cigarette; none was detectable in the smoke [22].

Little has been known of the daily exposure of man to nickel contained in ordinary foods. On a dry basis, lettuce, cabbage, spinach and peas were found to contain 1·5 to 3·0 p.p.m., while wheaten grain, potatoes and fruits had 0·15 to 0·35 p.p.m. [1]. HEGGEN and STOCK [23] found 2·2 to 3·0 p.p.m. nickel (dry weight) in leaves of poplar, oak, maple, fern and horsetails, and 5·2 p.p.m. in pine needles. They reported 0·03 to 0·06 p.p.m. (wet weight) in rat livers and 0·08 p.p.m. in pork liver. These results, by chemical concentration and spectrographic analysis, are comparable to ours.

Is nickel an essential mammalian trace metal?

If we define essentiality as having a normal physiological role and apply the working hypothesis expressed in previous reports [10, 14], nickel behaves like an essential trace metal for the following reasons: (1) It is ubiquitous on the earth's crust and in sea water. (2) It is present in plants and mammals. (3) It shows biological activity *in vitro*, affecting certain enzyme systems. (4) It has a low molecular weight and two interchangeable valences. (5) It is non-toxic to mammals orally except in astringent doses.

Nickel appears to be a biologically neglected metal. Probably more is known about its chelates than about those of any other metal [2]. It forms square planar and tetrahedral chelates common to most essential trace metals. Complexes with nitrogen–oxygen ligands are more stable than are those of any other transitional metal but copper [24]. Nickel, a siderophile element [15], has little affinity for sulfide, but behaves more strongly towards amino acids, serum albumin and pteridines than does zinc and cobalt [25]. Along with some other transitional metals, it can act as a bridge between pepsin and organic compounds or dyes, its stability constants being greater than cobalt, zinc and manganese, but less than copper and mercury [25]. It has the ability to displace beryllium from alkaline phosphatase and reactivate the enzyme [26]; it activates arginase *in vitro* (along with cobalt, iron and manganese [27]). No enzyme known requires it, although alone it catalyzes decarboxylation of some amino acids [28] and the saturation of unsaturated fats.

Nickel may have a physiological role for the following indirect reasons: (1) It is the only first transitional metal from atomic numbers 22 to 30 which has not been shown to have specific biological activity *in vitro* or *in vivo* [29]; (2) It is present in the newborn; (3) The existence of intestinal or hepatic barriers to absorption are implied; (4) It has little tendency to accumulate in tissues during a lifetime of exposure. Definitive experiments, however, have not been done.

KIKKAWA, OGITA and FUJITO [30] proposed an hypothesis, based on the strong affinities of melanin and its precursors for metals, that color depended upon specific metals which were transmitted as genes. To back up their hypothesis, they showed that in various samples of rabbit hair, black contained cobalt, copper and nickel; yellow nickel, titanium and molybdenum; and white, nickel. Experiments with melanin precursors *in vitro* produced white pigment with nickel; yellow with titanium; red with molybdenum and blue, green or blackish with copper, cobalt and iron. These relationships held for analyses of fish skin, bird plumage, moth wings (*Bombyx mori*), the eyes of fruit flies, guinea-pig hair and human hair, being less definitive in the last two. They also held for anthocyanins and carotenoids in plants (and in their bulbs and seeds), red being associated with iron, blue with molybdenum, white with nickel or copper, yellow with titanium. It is known that red hair has a high concentration of iron [31].

Further experiments briefly reported by KIKKAWA [32] are of interest. White-eyed drosophila mutants, white goldfish, white mice and white rabbits absorbed nickel from an environment containing several metals, while cobalt, molybdenum and cobalt were absorbed by brown-eyed drosophila, red goldfish and black mice and rabbits, respectively. The abnormal metals were either toxic, or in the case of mammals, were excreted in the urine. In respect to genetic transmission, ribonucleic acid from several sources contained nickel (as well as chromium and manganese) in constant ratios [33]; desoxyribonucleic acid can transmit at least two divalent cations genetically [34] and therefore both compounds may bind metals. The affinity of nickel for human epithelial tissue has been shown. Therefore, the evidence that nickel may play a role in pigmentation (or the lack of it) is quite suggestive, though indirect.

KERTESZ [35] has shown that plant polyphenol oxidase, a copper enzyme, can have tyrosinase activity when the copper is replaced by cobalt, vanadium and nickel. Copper can readily be removed, leaving the inactive apoenzyme, which is then reactivated by the metal ions. A further step, determining the color of the melanin resulting from such tyrosinase activity, has not been done. Likewise mammalian tyrosinase can give up its copper by dialysis, partial activity being restored by nickel, but not by other transitional metals [36]. Again, the color of the melanin resulting has not been reported.

Other indirect evidence for Kikkawa's theory lies in some older work reported by NOBLE [37]. A mineral mixture, composition unstated, "enormously increased the intensity of pigmentation" of beef-fed (nickel deficient from our data) *Ambystoma* larvae. Feeding of white worms to the red-bellied salamander prevented the development of the red color. Another variety of salamander reared on white worms and pale copepods developed a white instead of a yellow abdomen. Toad larvae raised on meat became intensely dark compared to others on a vegetarian (high nickel) diet. Fatty foods lightened amphibian skin. If nickel is the biological factor of whiteness, these experiments fall into line.

Nickel, however, was not shown to be a specific catalyst for oxidation of any of twelve natural substances and drugs examined by TANNER, unlike vanadium, cobalt, iron and manganese [38]. Its activity on the enzymes so far tested appears to be non-specific and common to several divalent metals. Other systems need to be examined for specificity.

If, as KIKKAWA showed, an organism not using nickel excretes it, differences in urinary nickel might be found in negroes and white persons. Urines of 12 African natives from Usumbura, Ruanda-Urundi, analyzed by PERRY and PERRY [19] had 180 μg per liter (range 27 to 500 μg), as compared to urine from 14 white St. Louisans who had a mean of 18·1 μg per liter. Unfortunately for this hypothesis, St. Louis negroes excreted little more nickel (mean, 23·4 μg per liter) than did white persons.

Apparent racial or local differences in the occurrences of detectable nickel (Tables 1 and 2) led to an examination of American tissues. There were no racial differences in intestine or skin. Nickel was present in 18·75 per cent of white and 32 per cent of negro livers; chi square distribution was barely significant at the 5 per cent level of confidence. For kidney, the distribution was similar, 27 and 45 per cent respectively, at less than the 10 per cent level of confidence. From Table 1, however, the frequency of occurrence in African livers is low, except in one location, Usumbura, and there are wide variations of nickel in livers and kidneys of other races, with means about double that of American tissues. The lack of a consistent trend in four tissues with color of skin is thus obvious. Considering the data in Table 2, these apparent differences are more explicable on geographical (or dietary) than on racial grounds.

SUMMARY AND CONCLUSIONS

Spectrographic data on the concentrations of nickel in human tissues at various ages and from various parts of the world were examined and a variety of foods, waters, animal tissues, vegetation and manufactured materials were analyzed by microanalytical methods in order to ascertain sources.

Nickel appears to be almost ubiquitous in vegetation and vegetable products, both edible and manufactured. High concentrations were found in several whole grains and legumes. A few processed foods apparently were contaminated during manufacture.

There was little or no nickel in most edible animal products, such as meat, milk and eggs. Water probably contributed little or none to the total bodily pool and air a minor increment. Average daily exposure on a normal diet was estimated at about 0·5 mg or less, of which about 5 per cent may appear in urine.

Nickel was found in the livers and kidneys of wild birds and animals, except for deer and red squirrel. Wild vegetation contained high concentrations and was the probable source.

Nickel was present in the human intestine at birth and its concentration remained fairly constant throughout life. It appeared to have a predilection for epithelium. In lung, unlike other tissues, its frequency and concentration increased with age. It was detected in only one-fourth to one-half of metabolically active tissues.

Available data suggests that there are geographical differences in the presence of nickel in tissues other than intestine from seven large cities of the United States, which also apply to kidney and liver. From nineteen other cities of the world analyses of kidney and liver show variations in frequency of occurrence and in concentration, which are largely unexplained other than on geographical bases.

From a theoretical viewpoint, what is known about nickel is consistent with the idea that it may play a physiological role in mammals and plants, possibly concerned with pigmentation. That role has not been proven.

Acknowledgments—The authors are indebted to PROFESSOR JUN KOBAYASHI for supplying samples of Japanese wheat and rice, and to Doctor HENRY L. HEYL for the salmon livers.

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J. Nutr 83:239-50(1964)

Chromium, Lead, Cadmium, Nickel and Titanium in Mice: Effect on Mortality, Tumors and Tissue Levels[1]

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ABSTRACT About 700 mice were given throughout their lives 5 ppm cadmium, lead, chromium, nickel or titanium in drinking water while fed a diet deficient in cadmium and low in other metals. Tissue concentrations comparable to those of man were observed. Sex differences appeared. Mortality of females was unaffected. Mortality of males on cadmium, lead and nickel was increased compared with that of the chromium group, and of those on cadmium and lead compared with the controls. Longevity of the oldest 10% of both sexes was less in lead and titanium groups, and of males in cadmium group, compared with controls. No metal was carcinogenic; incidence of tumors in males on cadmium and lead, and in females given nickel was decreased. Body weights at death were greater in titanium, chromium and lead groups. Large increments of titanium, moderate increments of cadmium and nickel, and lesser increments of chromium and lead were observed in 5 organs, compared with controls. Higher concentrations of cadmium and titanium occurred in younger mice and the other metals did not increase markedly in tissues with age. All metals except chromium exhibited one or more signs of innate toxicity.

We have reported the effects of small doses of chromium, lead, cadmium, nickel and titanium in drinking water on the growth and survival of mice up to 21 months of age in experiments attempting to duplicate human concentrations (1). Chromium and titanium increased growth rates in both sexes; in males chromium lessened early mortality, whereas cadmium and lead increased mortality at older ages. This report is concerned with our total experience for the lifetime of these animals, regarding mortality, gross causes of death, effects on incidence of tumors and organ accumulations of metals.

MATERIAL AND METHODS

The experimental conditions of the relatively metal-free environment has been reported in detail (1). White Swiss mice of the Charles River strain numbering 697 were exposed from the time of weaning to drinking water containing the essential trace metals, manganese, cobalt, copper, zinc and molybdenum. The diet of seed rye flour, powdered skim milk and corn oil was low in most of the metals under study and devoid of cadmium (1). It also contained only a trace of arsenic, 0.05 μg/g. Iron and vitamins were added. Plastic cages, stainless steel covers and drinking tubes, polyethylene water bottles and wood chips of low metallic content largely prevented environmental exposures to extraneous trace metals. One of the following as the acetate or oxalate[3] was added to the drinking water at 5 ppm metal: cadmium, divalent lead, trivalent chromium, divalent nickel and tetravalent titanium, and given for life to 50 or more animals of each sex. Experiments continued until all animals had died; the total elapsed time was 36 months. No animals were bred.

Dead animals were weighed and dissected, gross anatomical lesions were recorded and abnormal tissues fixed in Bouin's solution, sectioned and stained. Microscopic examinations were made on 257 sections. Although mice were autopsied as they died and each series was run concurrently, cannibalism and gross autolytic changes occurring over week-ends made many carcasses unfit for analysis. The figures shown in the tables, 251 males and

Received for publication December 7, 1963.
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222 females, are those of available autopsied mice, whereas data on mortality comprise all 697 animals.

Organs large enough for analysis (heart, lungs, kidneys, liver, spleen) were frozen in polyethylene bottles and, after a convenient interval, 3 to 6 of each group were pooled according to age at death, dried at 110°C, ashed in a muffle furnace at 400°C and analyzed for as many of the metals as the size of each sample allowed. Controls for each metal included tissues of animals given another metal. Microanalytical chemical methods of Saltzman (2, 3) and Sandell (4) were used as previously reported (5–9). Nearly 1000 analyses were made. All data are given in terms of wet weight and a value of 0.0 indicates that the metal was not detected by the method used. Statistics were analyzed by chi-square or Student's *t* test.

Water was weighed to the nearest gram at frequent intervals. Unavoidable wastage was caused by, 1) slight variations in environmental temperature, 2) movement of the drinking tube by playing mice, and 3) the habit of stuffing bedding, food and feces into the drinking tube with consequent irregular leakage. Food was allowed ad libitum and was not weighed because of considerable wastage in powdered form.

RESULTS

Major causes of death. The gross anatomical lesions in the 473 mice autopsied were divided into 4 categories: tumors, internal hemorrhage, infection and others, plus unknown (visible renal or hepatic lesions, splenomegaly, dilatation of the intestines or no major abnormalities). In table 1 are shown the causes of death. The only significant change was the reduction of visible tumors in a cadmium, lead or nickel group. In the control females there were 9 pulmonary, 3 mammary and 2 thyroid carcinomas, and one each in liver, peritoneal cavity, skin, ovary, ureter and of unknown origin with extensive metastases, as well as 2 leukemic infiltrations of spleen. Only 3 mice given nickel had tumors, all pulmonary ($P < 0.01$). Two to 5 mammary tumors and 4 to 5 lung tumors occurred in each of the other metal-fed groups.

In the control males there were 8 lung carcinomas, 1 sarcoma in the liver and 2 leukemic infiltrations of spleen. Only 2 tumors appeared in the lead and cadmium groups. One mouse given lead, one given titanium and none given cadmium had a pulmonary tumor. The incidence of these tumors was 13.5% in 222 autopsied females and 12.5% in 164 males, excluding

TABLE 1

Gross causes of death in mice[1] *given various metals: significant differences from controls*

	Controls	Cadmium		Lead		Nickel		Titanium		Chromium
	No.	No.	P value[2]	No.	P value	No.	P value	No.	P value	No.
Males										
Tumors	11(5)[3]	1	< 0.005	1(1)	< 0.01	7(4)		4(3)		6(3)
(Lung)	8	0	< 0.01	1		5		1	< 0.05	6
Hemorrhage	7	16		12		14		10		12
Infection	5	4		15		9		5		11
Other and unknown	21	27		11		11		21		10
Total no.	44	48		39		41		40		39
Females										
Tumors	22(8)	10(2)		11		3(3)	< 0.01	7(2)		9(4)
(Lung)	9	5		4		3		5		4
Hemorrhage	9	9		5		8		3		2
Infection	11	6		10		12		9		11
Other and unknown	18	14		3		10		13		7
Total no.	60	39		29		33		32		29

[1] Autopsied mice.
[2] P = probability according to chi-square analysis of differences from controls being due to chance.
[3] Numbers in parentheses are deaths from tumor before 600 days of age.

those given cadmium and lead. By chi-square analysis of these larger groups, the reduction of male pulmonary tumors associated with cadmium and lead was significant ($P < 0.005$).

The ages at death of the 30 male mice with tumors were studied to ascertain whether early mortality from cadmium or lead could affect the appearance of neoplasms. Whereas 16 deaths from tumor (53.3%) occurred before 600 days of age, one in the lead group died during this interval ($P < 0.05$). Of the 14 deaths from tumor after 600 days of age, one was in the cadmium group ($P < 0.05$). At the half-lives of each group none of the 8 deaths from tumors were in the cadmium- or lead-fed animals. At 500 days of age 46 males were alive in these 2 groups; tumors occurred in only two ($P < 0.05$). In the females 2 of the 21 deaths from tumor in the cadmium and lead groups occurred before 600 days of age, compared with 17 (27.4%) in the others ($P < 0.025$).[4] No deaths of nickel-fed mice occurred after 547 days of age ($P < 0.005$).

The typical lung tumor was an adenocarcinoma, of which 45 were observed out of 51 lung tumors. Eleven mammary tumors, 8 sarcomata, 4 splenomegalias of leukemic origin, 4 thyroid, 3 hepatic, 3 skin, 2 ovarian, 2 ureteral and 1 each in various other sites, totaled 92. The incidences of all tumors were: males 11.9%; females 27.8%.

There were no obvious changes in other causes of death, such as pneumonitis (19 cases), pyelonephritis (12 cases), mediastinal infections (4 cases), hepatic cystic or parenchymal degeneration (7 cases). Of 12 kidneys examined in the cadmium and lead groups, 8 showed hyalinized glomeruli, thickened basement membrane and a reduction of lumen to wall of arterioles; lesser alterations were seen in 3 others.

Mortality. Survival curves are shown in figures 1–6. Because the curves of 5 groups of male animals are rather straight, or slightly concave, unlike "normal" survival curves, indicating early mortality, a deficiency in the basic diet for males may have been present. As the rate of growth of mice fed chromium is increased (1) and the survival curve of males more nearly resembles a "normal" one, males fed each metal were compared with the chromium as well as to the control groups (figs. 3–6).

Compared with the chromium group, mortality rates were significantly higher in males given cadmium, lead and nickel. Males fed cadmium and lead also had higher mortalities at one or more intervals than did the controls. Significant differences in the groups on metals did not appear in females at any age (figs. 1, 2). Although mice of both sexes given titanium died at somewhat earlier intervals, changes were relatively small (figs. 2, 3).

To ascertain whether lifespan was affected, the ages at death of the last surviving 10% of animals in each group were compared with the controls. Longevity was less in each group fed a metal (table 2), and was significantly lower in males fed lead, cadmium, and titanium and in females given lead. Males receiving titanium lived 80.5%, cadmium 85.1%, chromium 86.8%, and lead 90.4% as long as controls, or 92 to 187 days less. Females given titanium and those receiving lead lived 91.5% as long as controls. There was no sex difference in mean lifespans of controls treated in this manner. A few animals lived more than 1000 days; 4 controls, three fed chromium and three given nickel. Of the titanium group the last surviving male died 231 days and the last surviving female 118 days before the oldest control animals. The relative order of decreased longevity was: males, titanium > cadmium > lead > chromium; females, titanium ≅ lead > cadmium.

Body weights at death. Mean weights of animals dying at less than one year, 1 to 2 years and more than 2 years of age were calculated. At ages after one year animals of both sexes taking nickel weighed somewhat less, by 4 to 13%, and those fed the other 4 metals, more than did the controls. The differences were small in females, 3 to 11%. In males dying under one year of age, mean weights compared with controls were: cadmium, 113%; lead, 122% ($P < 0.01$); chromium, 123% ($P < 0.025$); titanium 138%, ($P <$

[4] Percentages of deaths from tumor in both sexes occurring at various ages were: < 400 days, 3.1%; 400 to 499 days, 12.8%; 500 to 599 days, 23.4%; 600 to 799 days, 43.6%; 800+ days, 17.1%. The assumption is made that autolyzed and cannabalized mice not examined were randomly selected and did not influence the incidence of tumors.

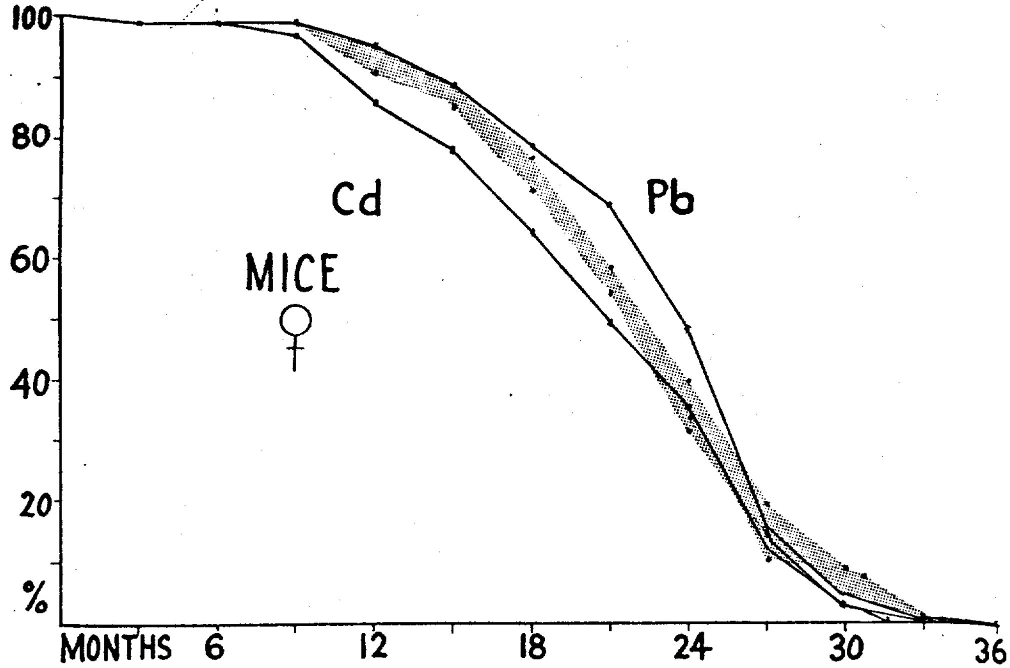

Fig. 1 Survival of female mice. The shaded area represents the curves of 54 mice given nickel, 54 given chromium, and 88 controls. Curves for 60 fed cadmium and 52 fed lead are shown separately. There were no significant differences at any 3-month period, except between lead and cadmium at 21 months, where $P < 0.05$ by chi-square analysis.

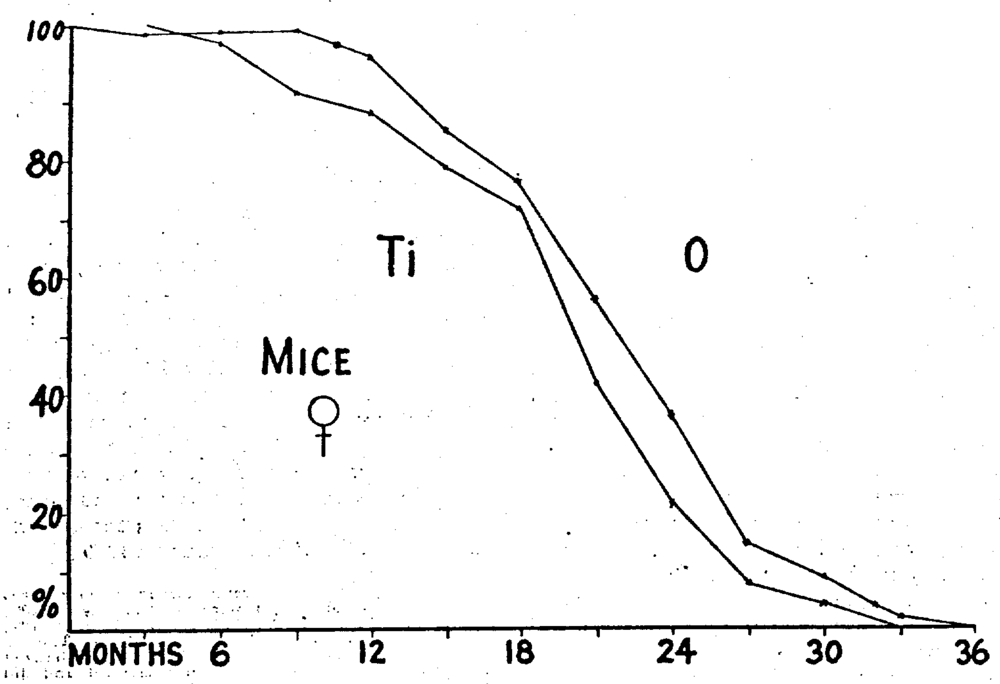

Fig. 2 Survival of 53 female mice given titanium and of 88 controls (O). No significant differences appear at any interval.

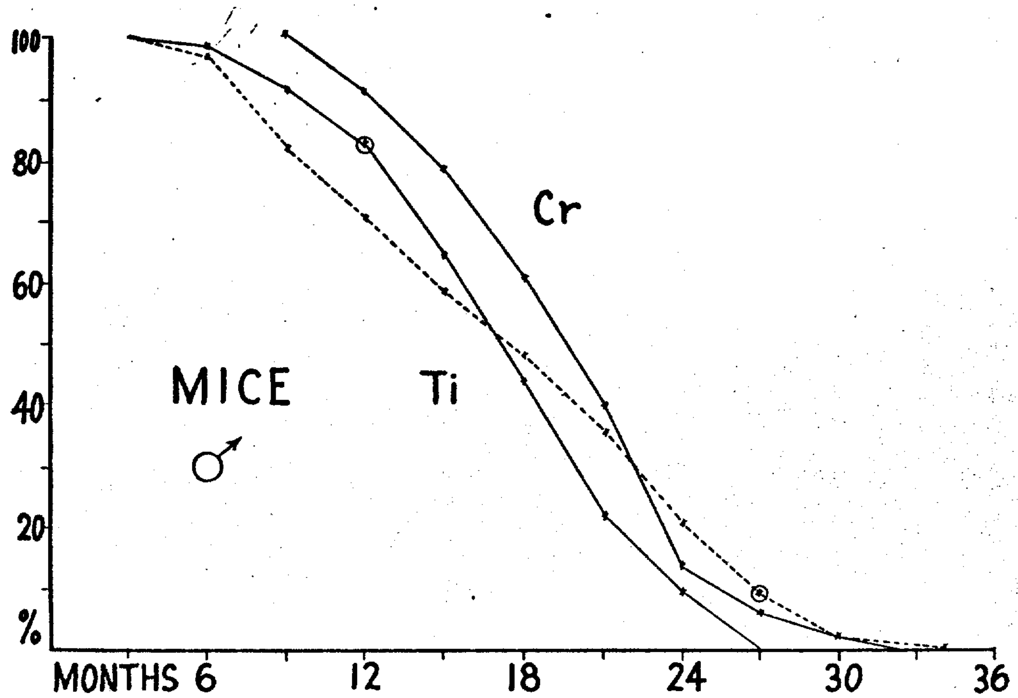

Fig. 3 Survival of 54 male mice given titanium, 54 given chromium and 61 controls (dashed line). The circles represent significant differences between titanium-fed animals and the controls; at 12 months, $P < 0.05$, at 27 months, $P < 0.025$. Chromium-fed mice differed from the controls significantly up to 17 months of age. Note that all given titanium had died by 27 months of age.

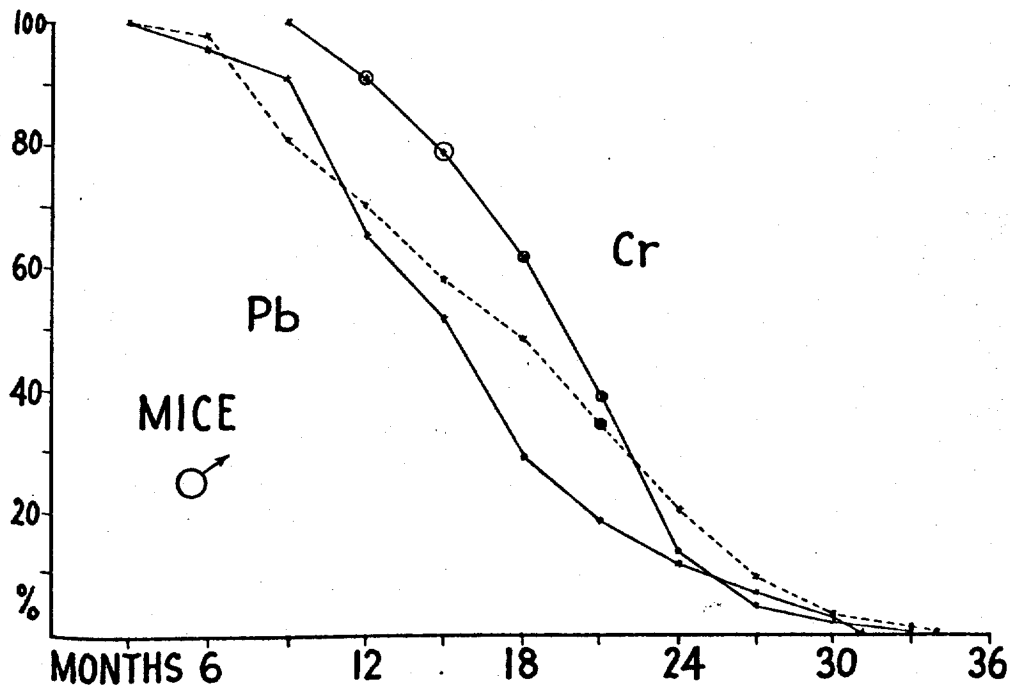

Fig. 4 Survival of 52 male mice given lead compared with 61 controls (dashed line) and 54 given chromium. The circles represent significant differences; at 12, 15 and 18 months, $P < 0.001$, at 21 months, $P < 0.025$. The half-life of lead-fed animals was 146 days less than those fed chromium.

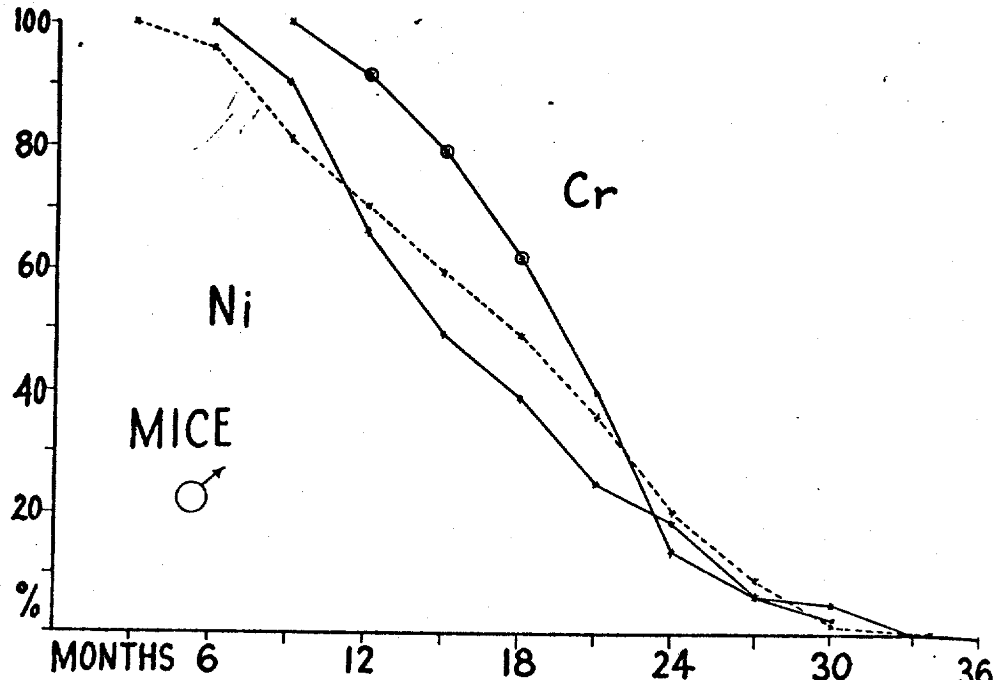

Fig. 5 Survival of 50 male mice given nickel, compared with 61 controls (dashed line) and 54 on chromium. The circles represent significant differences from the nickel-fed animals; at 12 and 15 months, $P < 0.0005$, at 18 months, $P < 0.02$. The half-life of the nickel group was 160 days less than that of the chromium group.

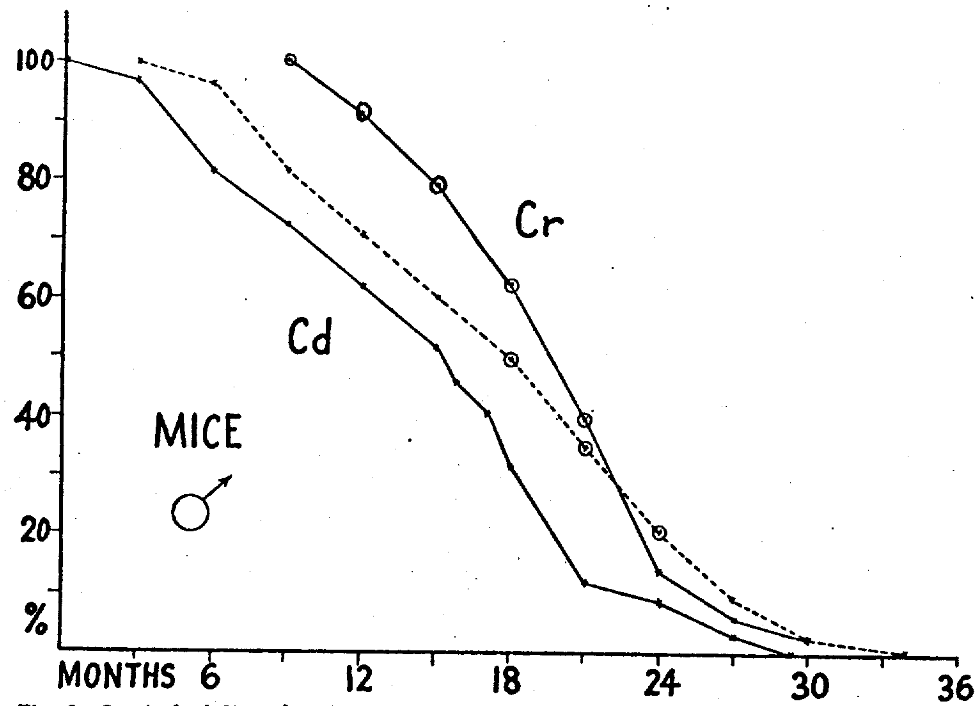

Fig. 6 Survival of 65 male mice given cadmium, compared with 61 controls and 54 fed chromium. The circles represent significant differences from the cadmium-fed animals; from 9 to 21 months, $P < 0.001$, for chromium; at 18 and 21 months for controls, $P < 0.01$; at 24 months, $P < 0.02$. Mice given cadmium had a half-life 133 days less than those fed chromium.

TABLE 2

Longevity of mice given trace metals: ages at which 75% were dead, mean ages at death of last surviving 10%, and ages of last survivors (days)

	Males				Females			
	No.	75% dead	Mean age	Maximal age	No.	75% dead	Mean age	Maximal age
Control	61	693	957	1035	88	777	966	1084
Nickel	50	623	896	995	54	750	929	1042
Lead	52	576	865	934	52	786	888	938
P value [1]			~ 0.025				~ 0.01	
Chromium	54	680	831	1016	54	740	940	1059
P value			< 0.05					
Cadmium	65	570	814	883	60	758	904	955
P value			< 0.005					
Titanium	54	618	770	804	53	702	884	966
P value			< 0.005				< 0.05	

[1] P is the probability of the difference from the controls being due to chance by Student's *t* test. The male titanium group also differed from the nickel ($P \sim 0.01$) and the lead ($P < 0.005$) groups. A few animals lived more than 912 days (30 months): males, 5 controls, 1 fed lead, 1 fed chromium, 2 fed nickel; females, 7 controls, 2 fed lead, 2 fed cadmium, 3 fed chromium, 4 fed nickel and 2 fed titanium.

0.005); and nickel, 103%. At 1 to 2 years of age, they were: chromium, 129%; nickel, 96% and titanium 109%, but differences were not significant at this or older ages.

Males in each group had lost 22 to 30% and females 28 to 37% of their body weights at the time of death. Relative weights, however, were of the same order as in living animals, those given titanium and chromium weighing the most and those given nickel the least (1).

Accumulation of trace metals. In tables 3–5 are shown the mean concentrations of the various metals in 5 organs according to sex, for mice 100 to 800 days or more of age, compared with values in the literature for adult man. At these low levels of dosage, no metal accumulated markedly with age; on the contrary, higher concentrations of cadmium and titanium appeared in younger mice. Table 6 indicates the levels in the organs of highest concentrations according to 3 ages.

Cadmium: Renal concentrations of 1.3 to 2.8 μg/g were observed in 4 males 134 to 174 days of age, 6.14 μg/g in 6 about one year of age, and 0.90 to 5.8 μg/g in 34 up to 2 years of age. In females the pattern was similar with a range of 0.86 to 6.5 μg/g. The mean renal concentrations at older ages was a third of that of younger mice. Cadmium was undetected in controls and in mice given other metals, except when animals raised commercially were analyzed, in which cases 0.01 to 0.3 μg/g were observed in kidney.

Lead: The food contained approximately 0.2 μg/g, an amount reflected in organ concentrations of controls almost always less than 1.0 μg/g; no accumulation with age was noted. Renal and hepatic ranges were 0.0 to 0.7 and 0.0 to 0.8 μg/g, respectively. Three groups of control mice were apparently devoid of lead. When given the metal, concentrations in spleen increased somewhat with age, but not in kidney (table 6). Males showed 0.52 to 3.3 μg/g and females 0.1 to 1.0 μg/g in liver, with 0.65 to 2.15 and 0.28 to 3.39 μg/g, respectively, in kidney. Thus there was some overlapping of values between controls and treated animals.

Chromium and nickel: These metals behaved similarly, with a predilection for spleen and heart and with little tendency to accumulate in other organs in amounts much greater than the controls. In male mice, concentrations of nickel in kidney and liver with ranges of 0.0 to 3.7 and 0.0 to 2.5 μg/g, respectively, decreased almost regularly from 300 to 755 days of age; in females the phenomenon occurred only in liver where the range was 0.0 to 2.3 μg/g. Taking chromium for 2 years did not cause significantly higher levels than one in any organ; the range of values in heart was 0.58 to 6.2 μg/g.

Titanium: High concentrations in liver and kidney were observed only in animals

TABLE 3

Organ concentrations of cadmium and lead in mice and man[1]

	No.	Kidney	Liver	Heart	Lung	Spleen	Mean[2]	Metal in food and water[3]
		µg/g wet wt	*µg/g wet wt*	*µg/g wet wt*	*µg/g wet wt*	*µg/g wet wt*		*ppm*
				Cadmium				
Mice								5.0
Males	44	2.94	0.63	0.20	0.34	0.66	0.93	
Females	35	2.65	0.66	0.62	0.42	0.27	0.92	
% present[4]		100	83	75	73	92		
Control mice[5]								0.0[6]
Males	69	0.0[6]	0.0	0.0	0.0	0.0	0.0	
Females	34	0.0	0.0	0.0	0.0	0.0	0.0	
Adult man	145	32.0	1.8	0.0	< 0.5	0.0	0.43	0.01
% present		100	98	3	50	13		
				Lead				
Mice								5.19
Males	41	1.25	1.37	1.03	1.17	1.42	1.25	
Females	23	1.35	0.39	0.81	0.49	2.68	1.14	
% present		100	83	92	67	90		
Control mice[5]								0.19
Males	118	0.51	0.37	0.58	0.33	0.53	0.46	
Females	121	0.27	0.25	0.82	0.33	0.70	0.47	
% present		81	69	80	67	92		
Adult man	145	1.2	1.5	< 0.05	0.67	0.27	1.10	0.12
% present		100	100	56	100	97		

[1] The levels for adult man are the means obtained by spectrographic methods from Tipton and Cook (10), subjects from the U.S. Mainland, and show general levels in tissues. The ranges in all cases were wide.
[2] The means of the averages for each organ are shown as indexes of relative tissue concentrations and are not to be construed as representing other than aids to the reader in comparing treated with control groups.
[3] Food and water values are measured levels for mice and approximations based on analyses for man (5–9).
[4] Percentage of organs analyzed in which the designated metal was detected.
[5] Controls represent all animals not given the metal indicated.
[6] No metal detected by analysis.

less than 260 days of age, with 3 to 15 µg/g in the young and 0.2 to 1.5 µg/g in the old. Heart, lung and spleen had much titanium, up to 32.5, 18.0 and 18.0 µg/g, respectively, in the young and up to 9.3, 6.7 and 9.9 µg/g in the older animals. Controls usually had little, 0.0 to 1.9 µg/g. In an attempt to establish a "standard," organs of wild field mice were compared; levels were mainly in the same range as laboratory mice given the metal, with a range of 0.4 to 7.98 µg/g.

Influence of one divalent metal on accumulation of another. Organ concentrations of a metal present in the basal diet were compared when another divalent metal was or was not given. Cadmium-fed animals had concentrations of lead in kidney, liver and lungs similar to controls; lead did not affect nickel in liver. Limitations in amounts of tissue prevented further such evaluations.

Intake of trace metals. There were no significant differences in the amounts of water ingested by the different groups. Because of variable amounts of unavoidable wastage, the total use varied from cage to cage as much as 14% in females and 25% in males from the means. Female mice took 3.86 and males 4.65 g/day. On a weight basis, mean use by males was 10.2 and by females 9.03 g/100 g/day, calculated during a representative year of adult life.[5] The fluid intake of male rats under identical conditions was 6.85 g and of females 7.51 g/100 g body weight/day.

As fluids contained 5 µg/g of metal, the intake including wastage could be roughly approximated at a maximum of 45 to 51 µg metal/100 g/day, or 16.48 to 18.62

[5] Use of water by single surviving males did not change in the several weeks or months before death; that by females usually doubled or tripled. Although the amount ingested could not be measured, there was no evidence that the intake of water and metal decreased in older animals.

TABLE 4

Organ concentrations of chromium and nickel in mice and man[1]

	No.	Kidney	Liver	Heart	Lung	Spleen	Mean[2]	Metal in food and water[3]
		μg/g wet wt	*μg/g wet wt*	*μg/g wet wt*	*μg/g wet wt*	*μg/g wet wt*		*ppm*
				Chromium				
Mice								5.1
Males	41	0.92	0.51	2.10	1.11	1.83	1.29	
Females	30	0.84	0.51	1.23	0.81	1.84	1.23	
% present[4]		100	100	92	91	81		
Control mice[5]								0.1
Males	33	0.38	0.09	1.04	0.39	0.40	0.46	
Females	30	0.34	0.15	0.31	0.17	0.42	0.28	
% present		75	65	74	70	40		
Adult man	145	0.01	0.007	0.015	0.13	0.005	< 0.09	0.04
% present		83	79	88	100	79		
				Nickel				
Mice								5.4
Males	33	0.93	0.78	1.05	1.13	2.76	1.33	
Females	27	1.07	0.75	0.72	0.53	4.16	1.45	
% present		71	78	75	70	91		
Control mice[5]								0.4
Males	69	0.52	0.62	0.43	0.32	0.42	0.46	
Females	30	0.46	0.20	0.0	0.61	0.33	0.32	
% present		100	87	67	67	75		
Adult man	145	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.14	0.2
% present		21	18	34	50	12		

[1] The levels for adult man are the means obtained by spectrographic methods from Tipton and Cook (10), subjects from the U.S. Mainland, and show general levels in tissues. The ranges in all cases were wide.
[2] The means of the averages for each organ are shown as indexes of relative tissue concentrations and are not to be construed as representing other than aids to the reader in comparing treated with control groups.
[3] Food and water values are measured levels for mice and approximations based on analyses for man (5–9).
[4] Percentage of organs analyzed in which the designated metal was detected.
[5] Controls represent all animals not given the metal indicated.
[6] No metal detected by analysis.

TABLE 5

Organ concentrations of titanium in mice and man[1]

	No.	Kidney	Liver	Heart	Lung	Spleen	Mean[2]	Metal in food and water[3]
		μg/g wet wt	*μg/g wet wt*	*μg/g wet wt*	*μg/g wet wt*	*μg/g wet wt*		*ppm*
				Titanium				
Mice								5.03
Males	41	2.86	1.81	8.80	4.81	6.83	5.02	
Females	37	2.89	2.05	4.10	1.66	3.70	2.88	
% present[4]		79	73	70	73	100		
Control mice[5]								0.03[6]
Males	31	0.33	0.38	0.34	0.13	0.94	0.42	
Females	51	0.55	0.67	1.08	0.66	1.10	0.81	
% present		57	56	75	50	67		
Wild field mice	9	1.03	4.10	6.93	3.03	—	3.02	
Adult man	144	< 0.05	< 0.05	< 0.05	2.2	< 0.05	< 0.21	0.12
% present		15	23	17	99	22		

[1] The levels for adult man are the means obtained by spectrographic methods from Tipton and Cook (10), subjects from the U.S. Mainland, and show general levels in tissues. The ranges in all cases were wide.
[2] The means of the averages for each organ are shown as indexes of relative tissue concentrations and are not to be construed as representing other than aids to the reader in comparing treated with control groups.
[3] Food and water values are measured levels for mice and approximations based on analyses for man (5–9).
[4] Percentage of organs analyzed in which the designated metal was detected.
[5] Controls represent all animals not given the metal indicated.
[6] Bedding (softwood chips) contained 2.31 μg/g.

TABLE 6

Trace metals in tissues of mice in relation to age, both sexes: organs of highest mean concentrations [1]

Metal	Target organ	100–360 days	360–560 days	560–800 days
			µg/g wet weight	
Cadmium	kidney	5.19	2.82	1.68
	liver	1.82	0.57	0.07
Lead	kidney	1.32	1.43	0.68
	liver	1.00	1.38	0.25
	spleen	1.11	2.04	2.18
Chromium	spleen	2.38	1.10	1.47
	heart	1.02	0.69	1.77
Nickel	kidney	1.85	0.78	0.57
	spleen	3.60	1.27	3.57
Titanium	spleen	7.59	6.64	1.49
	heart	10.05	3.60	3.30

[1] 5 ppm metal were given in drinking water continuously.

mg/100 g/ year. Therefore, even if half were wasted, the largest part of each metal ingested was not absorbed by body tissues (tables 3–5). The order of absorption, assuming that organ concentrations are representative, was: titanium >> nickel > lead > chromium > cadmium, and of the incremental differences from controls: titanium >> nickel ≃ cadmium > lead ≃ chromium.

Metals in newborn. The whole carcasses of 5 stillborn mice were analyzed. The results: cadmium 0.0, lead 0.0, nickel 0.14 and chromium 0.31 µg/g wet weight, indicating that 2 metals crossed the placental barrier and 2 did not. The mothers' tissues contained the metals.

Metals in tumors. Two lung tumors in mice given cadmium and 7 in controls showed none. Lead was observed in 8 of 10 tumors of control females at 0.08 to 2.1 µg/g. Nickel appeared in 2 of 3 tumors in control animals, chromium in two and titanium in 1 of 2.

Reproduction. No experiments were made on the reproductive capabilities of mice on metals. Eight hundred mice, however, have been successfully bred and raised with the cadmium-free diet, and analyses of representative groups failed to show tissue cadmium.

DISCUSSION

This exploratory study was designed to reproduce the lifetime human experience by causing accumulations of trace metals in mice equal to those of man (10) and comparing effects with animals little exposed to the same metals. We were successful in that all values observed lay within human ranges. In the case of lead, mean values were similar, but we produced less than mean human concentrations of cadmium and caused greater than mean human concentrations of nickel, chromium and titanium in both control and metal-fed animals.

Several unexpected effects were observed. There were marked sex differences in the responses of mice, males often being affected as to mortality and females not. At the 99% level of confidence, longevity of males was decreased by cadmium and titanium and of females by lead. Nickel appeared to decrease the incidence of tumors in females, and cadmium and lead in males. No metal was carcinogenic. Cadmium, lead and titanium, which accumulate in man with age, did not so accumulate in mice at the doses used; in fact, younger animals often had the highest levels at death. Although higher organ concentrations were observed in animals given metals, in all cases a state of "balance" appeared to be achieved independent of the duration of exposures; this phenomenon was especially marked in the cases of lead, chromium and nickel. Despite careful control of intake, the ranges of analytical results were wide and there were overlapping values for all metals except cadmium. We have found the analytical methods dependable and sensitive (5–9) and do not believe that methodological errors were of this magnitude. Individual differences in mice towards absorption and accumulation, therefore, may explain the variations in organ content of metals, early mortality being associated with high tissue levels. Similar variations occur in the tissues of man (10), which have been presumed to result from differences in exposure.

Duplications in mice of human levels of hepatic and renal lead of 1.5 ± 1.10 (SD) and 1.2 ± 0.87 μg/g wet weight, respectively (10), were associated with increased male mortality compared with controls and with mice given chromium, decreased longevity and fewer tumors. Lead in these concentrations, therefore, exhibits adverse effects on male mice not balanced by partial inhibition of tumors.

One-fifth to one-tenth of human renal cadmium levels of 32 ± 15 (SD) μg/g (10), in the range of children and African natives (11), were accompanied by decreased male survival and longevity despite suppression of tumors of the lungs. Deficiency of cadmium caused no obvious adverse effects on any parameter. Therefore, cadmium is not an essential micro-nutrient for mice but exhibits an innate toxicity in males at relatively low concentrations. In rats the major manifestation is hypertension (12, 13).

Nickel is not common in human tissues, except skin (10). Mice had higher levels whether fed it or not, although within human ranges. Whereas manifestations other than on female tumors were not obvious, nickel appears to have innate toxicity of an undefined nature, expressed by lessened male survival.

Chromium occurs in all human lungs but is not ubiquitous in other adult tissues; when present, it is only in small amounts (10). All but one of the mouse tissues analyzed had levels within the adult human extremes and resembled tissues of infants and children who have considerably larger concentrations (8). The improved growth and survival of both mice (1) and rats (14) at these levels, and the absence of adverse effects, suggest that chromium may have an essential function. Other evidence for its physiological role has been obtained (15).

Titanium had not been given chronically to animals, to our knowledge. Human lung contains a considerable amount, with a usual range of 0.55 to 7.0 μg/g and extremes up to 20 μg/g. Other tissues have little; therefore our mice resembled the human experiment in respect to lung but not to other organs. The unexpectedly large accumulations of titanium in laboratory and field mice suggest that homeostatic mechanisms are not active. Although improving the rate of growth (1), titanium is probably not an essential trace element because of its adverse effects on longevity.

Changes such as these here reported might occur sometimes in mice raised with various commercial diets which contain 0.4 to 1.9 ppm lead, 0.4 to 3.0 ppm nickel, 0 to 11.7 ppm titanium, 0 to 4.5 ppm chromium and 0.09 to 0.2 ppm cadmium (1). Our basal diet was low or deficient in these metals. One or more of them might affect growth, survival, longevity or tumors. If so, studies on mice should include control of the metal content of the diets given.

Comparison of our control data with those of Lindop (16) on a much larger series of SAS/4 mice reveal differences. The survival curve of our females, their median age and longest lifespan were somewhat less than hers, whereas body weights were greater. The incidence of tumors (38.6%) was also less (53.9%). Our control males had considerably shorter survivals, median age and longest lifespan than Lindop's and the incidence of tumors, 25%, was also less than the 41.0% reported by her. Whereas differing strains and diets probably account for these differences, the reduction in tumors in mice given metals is unlikely to be the result of these factors.[a]

These experiments demonstrate innate toxicities of lead, titanium, nickel and cadmium in male mice at tissue levels within human ranges, and a favorable effect of chromium. Low tissue affinity for chromium suggests that barriers to absorption may be present, as they may be in

[a] Although the incidence of tumors in inbred mice varies markedly with the strain (17), randombred animals in large enough numbers from the same source are not likely to show such great differences. The chance that 50 animals will have an incidence of tumors of 24% and another 50 or more obtained at the same time from the same source have 2% is small (< 0.01).

man (8), whereas such barriers are not as active for cadmium, lead, nickel and especially titanium. These experiments also indicate that in the oral doses employed, none of these metals is carcinogenic for mice.

ACKNOWLEDGMENT

We thank Dr. Kurt Benirschke for the microscopic sections and for his advice.

ADDENDUM

Since this report was submitted for publication, evidence has been obtained that rats on an identical regimen are partly deficient in chromium in respect to glucose metabolism.

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Book II Vacuum in tracer

Journal of Nutrition

104: 239-243, 1974

Life-term Effects of Nickel in Rats: Survival, Tumors, Interactions with Trace Elements and Tissue Levels[1,2]

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ABSTRACT In order to evaluate recondite toxicity of nickel, rats of both sexes were exposed to 5 ppm nickel in drinking water for life. The 104 rats given nickel and a control group containing 104 rats each received the following essential metals in water (ppm): zinc 50, manganese 10, copper 5, chromium 5, cobalt 1, molybdenum 1. There was some increased growth in the nickel-fed rats, but the metal was virtually innocuous, not affecting survival, longevity, incidence of tumors or specific lesions. Five organs were analyzed for zinc, copper, manganese, chromium, molybdenum and nickel by atomic absorption spectrophotometry. The feeding of nickel was associated with increased concentrations of chromium in heart and spleen, and manganese in kidney, and decreased copper in lung and spleen, zinc in lung, and manganese in spleen. Nickel did not accumulate in tissues. Uric acid levels in serum were unaffected. Nickel appeared to ~~interact~~ with all four of the essential trace metals studied. J. Nutr. *104*: 239–243, 1974.

INDEXING KEY WORDS trace minerals · trace metals · longevity · life span · mineral metabolism · mineral interactions

Effects of 12 trace elements given in low doses to rats for life have been evaluated in terms of growth, survival, longevity, tumors, tissue levels and serum constituents (1–4). The present report concerns nickel, a trace element essential for chicks (5, 6), which has recently been shown to have a physiological role in rats (7, 8).

Because one trace element may interact with others by displacement or competition, interactions of nickel with the essential metals zinc, copper, chromium and manganese were ascertained. Data from analyses of tissues of rats fed nickel compared to tissues of rats not given the metal were used to evaluate interactions. A number of unexpected interrelations were found.

METHODS

The environmental conditions of the "metal-free" laboratory and the design of life-term experiments have been described in detail (1, 9). Rats of the Long-Evans BLU:(LE) strain were born in the laboratory from random-bred pregnant females purchased from the supplier.[4] At weaning time litter mates were divided, four to a cage, in groups of 52 males and 52 females, between controls and treated animals. They remained in the same group of cages and on the same rack until natural death 2 to 4 years later. The diet was composed of 60% whole seed rye flour, 30% dried skim milk, 9% corn oil and 1% iodized sodium chloride, with added iron and vitamins (9).

Received for publication July 9, 1973.

[1] See NAPS document #02276 for 11 pages of supplementary material. Order from ASIS/NAPS, c/o Microfiche Publications, 305 E. 46th St., New York, N. Y. 10017. Remit in advance for each NAPS accession number $1.50 for microfiche or $5.00 for photocopies. Make checks payable to Microfiche Publications.

[2] Supported by Public Health Service Research Grant ES-00699-11, CIBA-GEIGY Corporation and Coca-Cola Laboratories, Inc.

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[4] Blue Spruce Farms, Altamount, N. Y.

TABLE 1

Weights of rats given nickel

Age, days	Control-3	Nickel
	g	
Males		
30	61±2.9[1]	68± 4.3
60	174±4.6	199± 7.3[2]
90	220±4.4	253± 8.1[2]
120	264±6.8	311± 9.2[3]
150	307±4.9	341± 9.6[4]
180	334±5.7	348±10.8
360	393±5.4	400±10.3
540	456±7.9	397±16.8[4]
Females		
30	70±2.6[2]	68± 3.5
60	150±3.9	158± 4.5
90	178±6.2	196± 4.3[5]
120	203±5.9	219± 5.7[6]
150	221±6.7	221± 5.1
180	234±6.2	229± 6.3
360	253±4.0	258±11.2
540	261±6.4	236± 8.4[5]
Intake, Ni, μg/g	0.44	5.44

[1] ±SEM. [2] Differs from preceding group at comparable age, $P < 0.01$. [3] $P < 0.001$. [4] $P < 0.005$. [5] $P < 0.025$. [6] $P < 0.05$.

Doubly deionized water from a forest spring was provided for washing cages and for drinking. To the drinking water were added as soluble salts the following metals (ppm): zinc 50, manganese 10, copper 5, chromium 5, cobalt 1, molybdenum 1 (9). This basal water was given to two groups of rats for life. A control group of 104 rats, designated control-3, was given the basal water and another group of 104 the basal water plus 5 ppm nickel and run concurrently.

Animals were weighed weekly for 3 months and then at monthly intervals. Dead animals and their hearts were weighed, and heart, lungs, liver, spleen and kidneys sectioned for microscopic examination and frozen for metal analysis. Tumors were weighed and sectioned. Sections were examined by light microscopy by two pathologists.

After drying to constant weight, pooled tissues were ashed in muffle furnaces at 400° in silica dishes; the ash was taken up in distilled nitric acid, diluted to 25 or 50 ml in deionized water and stored in polyethylene bottles. Tissues were pooled in groups of one to ten, usually less than five; subsequently, data for statistical evaluation were weighted according to the number of animals pooled.

Analyses for chromium, manganese, copper, zinc and nickel were made by atomic absorption spectrophotometry.[5] A three-slot Boling burner with air–acetylene flame was used for all elements other than zinc. For zinc, a nitrous oxide burner with air–acetylene flame was rotated 45° to the optical axis in order to reduce sensitivity. Sensitivities and precisions will be reported.[6]

Serum uric acid was measured on a spectrophotometer using premixed reagents and a method developed by the manufacturer of the instrument.[7] Weighted mean values were compared by Student's *t* and the sign test (α) (10), comparing the means of 10 pairs of male and female organs separately on a one-tailed distribution.

The diet contained (μg/g): molybdenum 0.25, nickel 0.44, cadmium 0.07, zinc 22.3, copper 1.36, manganese 12.7, chromium 0.14, cobalt 0.41. At an estimated food consumption of 6 g and a measured consumption of water of 7 ml/100 g body weight/day, daily intakes of nickel can be estimated at 2.6 or 37.6 μg for adult rats.

TABLE 2

Life spans of rats given 5 ppm nickel

Metal	No. rats	50% dead	75% dead	90% dead	Last survivor	Longevity[1]
				days		
Males						
Control-3	52	853	1004	1090	1160	1118±17.3
Nickel	52	857	952	1120	1162	1122±10.2
Females						
Control-3	52	872	1022	1149	1234	1177±28.4
Nickel	52	924	1079	1120	1346	1217± 7.4

[1] Mean ±SEM of last surviving 10%. Differences were not significant.

RESULTS

Growth rates. The rates of growth of rats given basal water and nickel are given in table 1. Nickel appeared to enhance growth at four age-intervals in males up to 6 months of age and two in females. At 18 months of age, nickel-fed rats were smaller than their controls.

[5] Perkin-Elmer 303, Perkin-Elmer HGA-70 Graphite furnace, The Perkin-Elmer Corp., Norwalk, Conn.
[6] Personal observations.
[7] Searle/BMI, 1220 Tenth Street, Berkeley, Calif. 94710.

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Use

TABLE 3

Gross and microscopic tumors in rats given nickel for life[1]

Metal	No. autopsied	Tumors No.	Tumors %	Sectioned No.	Tumors No.	Tumors %	Malignant No.	Malignant %
Males								
Control-3	40	13	32.5	23	9	39.1	4	17.4
Nickel	26	10	38.5	17	4	23.5	2	11.8
Females								
Control-3	35	18	51.4	26	10	38.5	7	26.9
Nickel	36	19	52.8	27	9	33.3	3	11.1

[1] There were no significant differences between controls and treated groups.

Life spans. Longevities and life spans are shown in table 2. No significant differences appeared.

Tumors. Compared to their controls, none of the groups fed nickel had an excessive number of gross, microscopic, or malignant tumors (table 3). The control-3 group had six sarcomata, two lymphomata and three carcinomata; the nickel-fed group had two carcinomata and three sarcomata. Benign tumors occurred as follows: six in the control and eight in the nickel group. No other specific lesions of organs were apparent in microscopic sections, except for a slightly increased incidence of focal myocardial fibrosis (13.3%) in the nickel-fed rats compared to the control group ($P < 0.025$).

Interactions of nickel with essential trace metals. Effects of feeding nickel on manganese, copper, chromium, and zinc in five organs of rats are given in table 4. There was some suppression of the levels of zinc and copper in lung and of manganese and copper in spleen. There was more chromium in heart and spleen, and more manganese in kidney, than in rats not fed nickel.

Accumulation of nickel. Table 4 also shows the concentrations of nickel in five organs of rats fed and not fed nickel. As much nickel from the diet was deposited in heart and spleen of rats given basal water as of rats fed nickel, indicating the lack of any tendency of nickel to accumulate.

Uric acid. Fasting serum uric acid in rats is usually low compared to man. Levels were (mg/100 ml): Males: control-3, 3.2 ± 0.35; nickel, 4.2. Females: control-3, 3.2 ± 0.76; nickel, 2.0.

Other changes. There were no obvious signs of depigmentation of these hooded rats fed nickel. Blanching of the incisor teeth, observed in many old rats, occurred in 17.3% of the control-3 group and 25.0% of the nickel group.[8] Weights of the hearts from the various groups did not differ significantly, nor did body weights at death.

DISCUSSION

It is clear from these data that nickel at 5 ppm was nontoxic when given to rats for life. No accumulation of nickel in tissues was observed. Nickel was not tumorigenic or carcinogenic, growth was enhanced, and survival and longevity were unaffected.

Nickel apparently interacted to some extent with all of the essential trace metals studied at the doses given in the following organs: lung, decreased zinc and copper; heart, increased chromium; kidney, increased manganese; spleen, increased chromium, decreased manganese and copper.

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In spite of a number of suggestions and indirect bits of evidence (11–13), proof of the essentiality of nickel for mammals has appeared only recently. Rats have thrived on 80 ppb nickel in the diet under strict isolation (14), as have quail (6). Chicks, however, have shown mild deficiency signs when given less than 79 or 40 ppb nickel in the diet (5). When refinement of the diet to 3 or 4 ppb nickel was accomplished, severe signs of deficiency in chicks were regularly produced; they included changes in shank skin color, a decrease in friability of the liver, decreased oxygen uptake by liver homogenates in the presence of α-glycerophosphate, increased lipids and decrease in phospholipids (7). Preliminary findings in rats were decreased viability of offspring, lethargy, reduced oxygen uptake by liver, decreased polysomes and increased monosomes in sucrose density gradients of

[8] Personal observations.

TABLE 4

Trace metals in tissues of rats fed nickel, dry weight, both sexes combined

Organ	Control-3 μg/g	P[1]	Nickel-fed μg/g	Control-3 μg/g	P[1]	Nickel-fed μg/g
		Copper			Zinc	
No. rats/no. samples[2]	(22/7)		(63/10)	(22/6)		(64/10)
Liver	22.8 ±4.88[3]	—	20.7 ±7.09	142 ±12.8	—	94 ±20
Lung	15.2 ±1.28	<0.01	9.8 ±0.97	80 ± 5.4	<0.05	60 ± 5.7
Heart	22.7 ±5.29	—	32.8 ±7.39	117 ±11.0	—	99 ±14.0
Kidney	22.1 ±6.58	—	27.0 ±5.76	108 ± 9.2	—	128 ±19.8
Spleen	24.2 ±2.75	~0.25	16.3 ±2.24	124 ±11.7	—	100 ±10.2
α[4]		—			0.01	
Intake in food & water	6.36		6.36	72.3		72.3
		Chromium			Manganese	
No. rats/no. samples[2]	(29/8)		(64/10)	(24/10)		(63/10)
Liver	0.51±0.078	—	0.41±0.061	4.5± 1.04	—	4.0± 0.87
Lung	2.03±0.738	—	1.04±0.375	1.8± 0.33	—	1.8± 0.28
Heart	0.61±0.100	<0.025	1.86±0.406	2.7± 0.46	—	3.1± 0.73
Kidney	0.94±0.270	—	1.48±0.247	2.2± 0.52	<0.025	4.2± 0.79
Spleen	1.11±0.355	<0.05	2.97±0.679	3.2± 0.59	<0.025	1.8± 0.40
Intake in food & water	5.14		5.14	22.7		22.7

Organ	Control-3 μg/g	P[1]	Nickel-fed μg/g
		Nickel	
No. rats/no. samples[2]	(34/9)		(64/10)
Liver	1.3±0.12	—	1.2±0.20
Lung	2.4±0.53	—	2.7±0.52
Heart	3.0±0.94	—	3.2±0.39
Kidney	1.7±0.46	—	3.0±0.70
Spleen	6.2±1.83	—	4.9±0.87
Intake in food & water	0.14		5.44

[1] P is the significance of the difference between contiguous mean values, by Student's t. [2] Numbers in parentheses are total number of rats/number of pooled samples. Organs were pooled in groups of one to ten; more than half were in groups of three to five. [3] Weighted mean ± SEM according to number of rats and samples in each pool. Note: Data on the two sexes separately, as well as on both sexes combined, have been filed with the National Auxiliary Publications Service of the American Society for Information Services. There was more chromium in the livers of females fed nickel than of males, and less in male lungs than control-3 ($P < 0.05$). There was more zinc in control-3 male livers than nickel-fed males ($P < 0.01$), and more nickel in plain water male livers than in nickel-fed ($P < 0.05$). A dash indicates $P > 0.05$.

liver (7). Therefore, nickel appears to be essential for chicks and probably for rats.

Three of our findings are consistent with nickel being an essential trace metal: a) growth enhancement, b) lack of toxicity, and c) lack of accumulation in tissues in spite of constant feeding. These findings are not definitive, but offer indirect evidence. In our experience, no essential trace metal given in low doses for life is toxic or accumulates in tissues (7).

Theoretically, metals can interact in the body in several ways: by competing for transport mechanisms or carriers in the gastrointestinal tract, by competing at the site of action, e.g., on enzymes or coenzymes, by promoting excess storage or release therefrom, and by promoting increased or decreased excretion. Analysis of tissues can detect such interactions, although not delineate them. Metals can also interact by combining in a stable complex, or by altering binding sites in proteins. Analysis of metal content of organs will not detect such interactions.

From the data, it appears that nickel mobilized and promoted the excretion of copper, zinc and manganese from one or two organs, and promoted storage of chromium and manganese in one or two organs.

These experiments lend further credence to the idea that most essential trace metals interact in the body to one degree or another, whether or not their atomic structures and radii are similar. The exact mechanisms are unknown.

ACKNOWLEDGMENTS

We are indebted to John B. Blennerhassett, M.D., Pathology Department, University of Otago Medical School, Dunedin, New Zealand and to Logan M. Mahaffey, M.D., Department of Pathology, University of Rochester Medical Center, Rochester, New York 14620 for examination of the microscopic sections.

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Farmakol. i Toksikol. 23:549-557, 1960 (No. 389)

TOXICITY AND DISTRIBUTION OF FINELY DISPERSED METALLIC NICKEL IN THE BODY

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Published data on the toxicology of nickel are sparse and conflicting. N. N. Bulatov found in experiments on frogs that nickel sulfate and nickel chloride are highly toxic. As little as 5 mg of nickel sulfate injected subcutaneously killed the experimental animals. Ceresa repeatedly injected this compound but did not observe any toxic effects. According to Eayolle and Canal, 10 to 20 mg/kg of soluble nickel salts (in metallic nickel equivalent) was a fatal dose for dogs when injected intravenously.

The information regarding the distribution of nickel in organs and tissues is highly contradictory. Royer noted that after parenteral injection nickel spread throughout the body and was deposited in nervous and muscular tissues, lungs, and, to a lesser degree, liver and spleen. According to Eayolle and Canal, the use of nickel chloride results in its accumulating in the liver and kidneys; when ingested, nickel is deposited in the bones, spleen, kidneys, heart, intestines, blood, and testes. The substance is excreted mainly with feces.

There are no published data on the toxicity, distribution, and excretion of metallic nickel.

The purpose of our work was to study the systemic action, toxicity, distribution, and excretion of finely dispersed metallic nickel after various modes of administration. We began with preliminary experiments to determine the toxicity of metallic nickel in different nickel compounds. We used metallic nickel particles 0.19 μ in diameter, soluble nickel sulfate and nickel nitrate, and poorly soluble nickel carbonate.

The experiments were performed on 145 white mice injected once intravenously with the compounds under study to prevent their being deposited at the injection site. The results are shown in Table 1.

It is evident from Table 1 that readily soluble nickel sulfate and nickel nitrate were the most toxic and metallic nickel the least toxic. The toxicity of the soluble compounds was 17.2 to 93.7 times higher than that of metallic nickel. This difference is probably due to the difference in degree of dissociation of the preparations under study. Our assumption is confirmed by the fact that the poorly soluble nickel carbonate occupies an intermediate position with respect to toxicity.

Table 1

Comparative Toxicity of Different Nickel Compounds Following a Single Intravenous Injection of White Mice

1 - Nickel compound

2 - Number of animals

3 - Minimum lethal dose of nickel (in mg/kg) in metal equivalent

4 - Time of death

5 - Absolute lethal dose (in mg/kg) in metal equivalent

6 - Time of death

7 - Metallic nickel (particle diameter 0.19 μ)

8 - 7th day

9 - Immediately after injection

10 - The same

11 - 5th to 15th days

12 - 2nd to 10th days

In a second series of experiments involving 80 white mice, 55 rats, and 15 rabbits, we studied the toxicity and systemic action of metallic nickel after repeated administration. A suspension of the preparation in distilled water was injected intravenously at doses ranging from 10 to 600 mg/kg daily for 5 days. The animals were observed 3 months. Toxicity was judged from the survival rate, general condition of the animals, and some clinical parameters (weight, temperature, blood morphology, permeability of skin capillaries,* composition of urine). In addition, the organs of some of the dead animals were [redacted] examined pathomorphologically. The distribution of nickel in the organs as well as its content in urine and feces were determined by emission spectral analysis(Table 2).

Table 2

Survival Rate of Animals After Repeated Intravenous Injection of Metallic Nickel (Particle Diameter 0.19μ)

1 - Animals
2 - Total dose (in mg/kg)
3 - Number of experimental animals
4 - Of these
5 - survived
6 - died
7 - Time of death (in days)
8 - 22nd and later
9 - Rats
10 - Mice

[redacted]

Table 2 shows that the minimum and absolute lethal doses for mice with this mode of administration ~~is~~ are 100 [redacted] and 600 mg/kg.

The minimum lethal dose was somewhat lower for rats, 50 mg/kg. Rabbits proved to be the most sensitive, the absolute lethal dose being 250 mg/kg.

A comparison of the results of the experiments on the white

*Permeability was studied by Bogdanova's method in Ivanitskiy's modification.

mice with [redacted] single and repeated injection of metallic nickel showed that the minimum and absolute lethal doses were similar in both cases. The differences did not exceed 25 to 100%. This suggests that metallic nickel has cumulative properties, unlike its soluble salts which do not (Ceresa, Ya. M. Grushko, V. A. Donskov, V. S. Kolesnik, and others).

The clinical picture of injury caused by metallic nickel was the same in the various animal species. When injected with large, lethal doses (500 to 600 mg/kg for rats and mice, 250 mg/kg for rabbits), the animals became sluggish and very thirsty. The rabbits' consumption of water increased [redacted] between days 2 and 3 from 50-100 to 150-200 ml a day. Breathing became labored with the intercostal muscles participating. Body temperature rose 1 to 2°. Albumin and sugar appeared in the urine from day 4. Most of the experimental animals died during the first 5 to 15 days.

The symptoms were less pronounced when smaller doses were used. The animals died later, on days 22, 35, 70 and thereafter.

A loss of body weight AS A FUNCTION OF [redacted] the dose was characteristic of all the experimental animals. In rats, for example, the weight lag on day 5 after a dose of 500 mg/kg was 30 to 40 g and constituted 20% of the initial weight (Fig. 1); after 200 and 50 mg/kg, it was 17 and 10%, respectively.

Fig. 1. Change in weight of rats after repeated intravenous injection of metallic nickel (mean data for each group of animals). 1 - control; 2 - 25 mg/kg; 3 - 50 mg/kg; 4 - 100 mg/kg; 5 - 200 mg/kg; 6 - 500 mg/kg.)

Study of skin capillary permeability showed that the rate of resorption of the fluorescein spot gradually increased with length of time after the start of the experiment. It was highest between days 10 and 30, reaching a level of 80 to 100% an hour compared to 30 to 40% an HOUR [redacted] in the control. The increase in permeability was the more pronounced and it set in earlier the larger the dose of nickel received by the animals.

The content of erythrocytes and hemoglobin concentration in peripheral blood did not change significantly. The leukocyte count also remained constant initially, but between days 6 and 15 SEVERAL ANIMALS developed a neutrophilic leukocytosis of about 15,000 to 20,000 leukocytes in 1 ml of blood.

Pathomorphological examination of the animals that died between days 3 and 8 after the injection of nickel showed the trachea and [redacted] large bronchi to be hyperemic.* The lung parenchyma was uneven in color and consistency: dense grayish foci alternated with pink-red areas.

*The histological examination was [redacted] carried out with the advice of senior scientist A. P. Novikova to whom we express our thanks.

Histological examination revealed marked perivascular edema, hyperplasia of peribronchial lymphoid tissue, and signs of bronchial-desquamative pneumonia. Lumps of dark brown pigment (Fig. 2) could be seen in the interalveolar septa and along the pulmonary blood vessels. The capillaries were markedly dilated and there were hemorrhages in the heart and brain, hyperemia and focal swelling of the epithelium of the convoluted tubules, and hemorrhages in the renal stroma. Reticuloendothelial cells in organs rich in reticular tissue (liver and spleen) were swollen and contained tiny granules of dark brown pigment.

Fig. 2. Presence of nickel in the pulmonary blood vessels of a rat that died 5 days after daily intravenous injection of the preparation in a total dose of 300 mg/kg.

In the animals that died between days 22 and 35 of the experiment, histological examination revealed not only acute exudative changes in the lungs but symptoms of productive inflammation in the form of hyperplasia of bronchial epithelium and thickening of the interalveolar septa (Fig. 3).

Fig. 3. Lungs of a rabit that died on day 22 of the experiment after intravenous injection of metallic nickel in a total dose of 50 mg/kg. Dilatation of the capillaries, focal edema, start of formation of granulomas around THE nickel deposits.

If an animal survived, its general condition improved 30 to 40 days from the start of the experiment and the signs of intoxication diminished, but [redacted] it did not fully recover. Later stages were marked by early alopecia and development of subcutaneous abscesses.

The scope of our study did not include special experiments to elucidate the clinical picture of poisoning by [redacted] soluble nickel compounds. However, the literature indicates that such poisoning occurs acutely and is characterized by spasms, vomiting, diarrhea, salivation, and distinct involvement of the centers regulating respiration and cardiac activity. We did not observe these symptoms in our experiments. The most prominent phenomena were lesions of the respiratory organs accompanied by disturbances in other viscera.

The tiny particles of metallic nickel [redacted] seem to be the major factor in the mechanism of action of the element. On penetrating into various organs and tissues they cause local disorders - hyperemia, edema, and other signs of inflammation - which give rise eventually to systemic disturbances. The local effect is naturally manifested most strongly in the regions where nickel is deposited. The question arises of whether the preferential involvement of the lungs is due to the intense irritation of this organ as a result of the substance accumulating here.

Spectroscopic study of the distribution of nickel in the body confirmed the correctness of this assumption. Table 3 presents data characterizing the nickel content of organs and tissues following intravenous injection of different doses of the preparation.

Table 3

Nickel content of organs in milligrams per 100 g of tissue following intravenous injection of finely dispersed metallic nickel

1 - Survival time of animals after poisoning (in days)
skipped ~~2~~ -
3 - Total dose used (in mg/kg)
4 - Lungs
5 - Kidneys
6 - Liver
7 - Spleen
8 - Heart
9 - Brain
10 - Small intestine
11 - Large intestine
12 - Muscles
13 - Stomach
14 - Bone
15 - Rats
16 - Not found
17 - Rabbits
18 - Traces

Table 3 shows that the [redacted] nickel content was HIGHEST [redacted] in the lungs during the first 5 days from the time the preparation was first used, and it constituted about 15 to 20% of the amount of nickel injected.* From 0.1 to 0.5% of the dose injected was found in the kidneys, liver, spleen, heart, and brain. On days 6 and 7 the nickel content of the lungs decreased 5- to 6-fold, an indication of the pronounced desorbing capacity of this organ. Such a decrease did not occur in other organs. In some animals, the nickel content of the kidneys even increased slightly at this time. However, in all cases the lungs remained the richest in the substance.

Nickel began to be excreted on days 2 and 3 of poisoning, the peak occurring on day 7. The intensity of the process diminished sharply thereafter and practically no nickel was found in the excretions after 9 or 10 days. The amount excreted with feces exceeded [redacted] that excreted with urine several times.

Fig. 4 shows a characteristic curve of nickel excretion from a white rat after intravenous injection of 500 mg/kg of the preparation.

Fig. 4. Curves of nickel excretion with feces (1) and urine (2) from a rat that received 500 mg/kg of metallic nickel intravenously.

We ran special experiments to determine the nature of the retention of [redacted] nickel by lung tissue. We wanted to learn which is more significant: sorption of finely dispersed metallic nickel by the very developed lung surface or affinity of nickel for lung tissue. Accordingly, we studied the distribution of nickel in lung tissue.

*The rats' and rabbits' lungs weighed on the average 1.5 g and 20 g, respectively.

organs and tissues following the administration of its soluble nitrates, sulfates, and carbonates (ionic forms of Ni). During the first 3 days nickel accumulated in the kidneys, liver, lungs, spleen, and heart. However, by day 6 most of it was found in the lungs as a result of redistribution. These observations implied some affinity of nickel for lung tissue. This was also suggested by an examination of the organs and tissues of rabbits not poisoned with nickel. The content of this trace element in the lungs constituted about 0.016 mg per 100 g of tissue. It could not be detected in other organs by the method used.

We thought it interesting to find out whether the behavior of finely dispersed metallic nickel remains more or less the same regardless of the route of entry. Accordingly, we ran a third series of experiments involving peroral administration of 50 to 1200 mg/kg to 70 white mice and 55 rats.

Peroral
[redacted] administration at doses of up to 100 mg/kg did not have any significant effect; 200 to 400 mg/kg caused sluggishness and loss of weight while the body temperature remained constant. A dose of 500 mg/kg was the minimum lethal dose for both species of animals. The median lethal doses calculated by Berens' method were 940 and 780 mg/kg for mice and rats, respectively. Most of the animals died [redacted] 3 to 5 days after the administration of lethal doses.

Dissection of the animals revealed the stomach and intestine to be somewhat hyperemic, distended, filled with food, and black. The mesenteric blood vessels were dilated. No gross changes were visible in other organs.

In this series of experiments, the distribution of nickel differed from that observed after intravenous injection of the preparation.

Table 4

Nickel content of organs in milligrams per 100 g of tissue in rats following peroral administration at a dose of 750 mg/kg

1 - Survival time after poisoning (in days)
2 - Lungs
3 - Kidneys
4 - Liver
5 - Spleen
6 - Heart
7 - Brain
8 - Muscles
9 - Bones
10 - Traces

Table 4 shows that the largest amount of nickel during the first 3 days after administration was present in the kidneys, liver, lungs, spleen, and heart, constituting about 0.5% of the dose injected. On day 6 there was a redistribution of nickel along with an increase in the absolute amounts in the various organs and tissues. The lungs were the most saturated with nickel at this time.

The bulk of the nickel was not absorbed and it was excreted with feces.

CONCLUSION

Our experiments showed that finely dispersed metallic nickel has a pronounced toxic effect after repeated administration, especially after intravenous injection. In the case of the latter, the minimum toxic and minimum lethal doses [redacted] were, respectively, 10 and 50 mg/kg and 25 and 100 mg/kg.

Of the animals we studied, rabbits were the most sensitive to nickel and mice the least. [redacted]

Most of the experimental animals that received lethal doses died within 5 to 15 days. The cause of death was pneumonia accompanied by degeneration of the parenchymatous organs.

After intravenous injection, most of the nickel was found in lung tissue. This appears to be the etiological factor in the development of pneumonia. However, the entry of nickel into other organs, as manifested by changes in the liver, kidneys, heart, and and other organs, may also be significant in the general picture of intoxication.

It is interesting to note that we also detected the seledtive accumulation of nickel in the lungs [redacted] somewhat earlier in experiments on rabbits with intratracheal insufflation of finely dispersed metallic nickel (these animals too died of pneumonia).* We ascribed this to the barrier function of lung tissue. The individual particles are very small (less than 1μ in diameter) and the dust penetrates directly into the blood through the lungs after mixing with air and forming an aerosol.

Our experiments indicate that intratracheal administration may also result in secondary accumulation in the lungs of nickel transported there with blood.

When metallic nickel enters the body through the mouth, it is less toxic, causing local changes in the gastrointestinal tract (hyperemia). Doses ranging from 500 to 1200 mg/kg kill the animals. With this mode of administration most of the nickel is excreted with feces. Hence its content in the organs in our experiments was much less than after intravenous injection when it constituted about 0.5% of the dose used.

The distribution of nickel after peroral administration during the first few days of the experiment differed from that after intravenous injection. Most of the nickel accumulated in the kidneys, liver, and lungs. However, by day 6 it was redistributed, the amount increasing in the lungs. The same distribution occurred after the administration of the soluble salts. This shows that after peroral administration metallic nickel partly dissolves in the gastrointestinal tract and is absorbed in the form of soluble salts.

SUMMARY

1. Metallic nickel with particles 0.19 in diameter had a pronounced toxic effect on rabbits, rats, and white mice after repeated intravenous injection. The range between the minimum toxic and absolutely lethal doses was from 10 to 600 mg/kg for rats and mice. The absolutely lethal dose for rabbits was 250 mg/kg.

2. The toxic effect of metallic nickel after peroral administration was less pronounced. The minimum and absolutely lethal doses for rats and mice were 500 and 1200 mg/kg, respectively.

3. Nickel has an affinity for lung tissue. Following intravenous injection, the content of metallic nickel was highest in the lungs throughout the experiment, a factor conducive to the development of pneumonia.

After peroral administration, the distribution of the absorbed part of nickel was analogous to that of its soluble salts. During the first 3 days it accumulated in the kidneys, liver, lungs, and spleen. On day 6 of the experiment, it was most abundant in lung tissue due to redistribution.

4. Most of the nickel was excreted the first 7 days. Excfetion of the element with feces exceeded that with urine. After intravenous

*The results will be reported in a separate communication.

injection, the difference was 400 to 600%.

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4. Гинк и его производные инактивируются в организме кроликов в значительных количествах путем разрушения этих препаратов до веществ, не дающих реакции на гидразиновую группировку.

5. У собак ГИНК и его производные инактивируются в небольшом количестве и выделяются с мочой в форме веществ, обладающих бактериостатическими свойствами.

Поступила в редакцию 28/XII 1959 г.

Farmakol. i Toksikol, (389)23: 549-557 (1960)

ТОКСИЧНОСТЬ И РАСПРЕДЕЛЕНИЕ В ОРГАНИЗМЕ МЕЛКОДИСПЕРСНОГО МЕТАЛЛИЧЕСКОГО НИКЕЛЯ

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Литературные сведения о токсикологии никеля немногочисленны и противоречивы. Н. Н. Булатов в опытах на лягушках установил высокую токсичность сернокислой и хлористой солей никеля. По его данным, уже 5 мг сернокислого никеля, инъецированные подкожно, вызывали гибель подопытных лягушек. Черас (Ceresa) при многократном введении данного соединения не обнаружил токсического эффекта. По сообщению Кожоля и Каналя (Kayolle, Canal), при внутривенном применении собакам растворимых солей никеля летальной дозой оказались 10—20 мг/кг в пересчете на металлический никель.

Относительно распределения никеля в органах и тканях имеются самые разноречивые сведения. Руайе (Royer) отмечает, что при парентеральном введении никель распространяется по всему организму и откладывается в нервной и мышечной тканях, в легких и в меньшей степени — в печени и селезенке. По сообщениям Кожоля и Каналя, применение хлористоводородной соли никеля ведет к накоплению его в печени и почках, при введении внутрь никель задерживается в костях, селезенке, почках, сердце, кишках, крови и семенниках. Выделение этого вещества производится главным образом с калом.

Сведения о токсичности, распределении и выведении из организма металлического никеля в литературе отсутствуют.

Целью нашей работы являлось изучение общего действия, токсичности, распределения и выведения из организма мелкодисперсного металлического никеля при различных способах применения.

Исследования мы начали с предварительных опытов, в которых определяли токсичность металлического никеля в ряду различных соединений никеля. Для изучения были взяты металлический никель, имевший размер частиц 0,19 μ, растворимые сернокислый и азотнокислый никель, а также плохо растворимый углекислый никель.

Опыты проводили на 145 белых мышах, которым однократно внутривенно инъецировали исследуемые препараты, что исключало возможность депонирования их на месте введения.

Результаты этих экспериментов представлены в табл. 1.

Из табл. 1 видно, что наиболее токсичными в наших опытах оказались хорошо растворимые сернокислая и азотнокислая соли никеля и наименее — металлический никель. При этом токсичность растворимых соединений была в 17,2—93,7 раза выше, чем металлического никеля. Разницу в токсичности по всей вероятности следует объяснить различной степенью диссоциации испытуемых препаратов. Подтверждением этого предположения является тот факт, что трудно растворимая соль углекислого никеля занимает по токсичности среднее место.

Во второй серии экспериментов, проводившейся на 80 белых мышах, 55 крысах и 15 кроликах, изучали токсичность и общее действие металлического никеля при многократном его поступлении в организм. Испытуемый препарат в виде взвеси в дистиллированной воде инъецировали внутривенно в дозах от 10 до 600 мг/кг ежедневно в течение

Таблица 1

Сравнительная токсичность различных соединений никеля при однократном внутривенном введении белым мышам

Соединения никеля	Число животных	Минимальная смертельная доза никеля (в мг/кг) в пересчете на металл	Срок гибели	Абсолютно смертельная доза (в мг/кг) в пересчете на металл	Сроки гибели
Металлический никель (размер частиц 0,19 μ)	45	50	7-й день	450	5—15-й день
$Ni_3(OH_2)CO_3$	10	—		380	2—10-й день
$NiSO_4$	45	2,9	Сразу после введения	4,8	Сразу после введения
$Ni(NO_3)_2$	45	2,9	То же	4,8	То же

5 дней. Наблюдение за животными длилось в течение 3 месяцев. О токсическом действии судили по выживаемости, общему состоянию животных и по некоторым клиническим показателям (вес, температура, морфологический состав крови, состояние проницаемости капилляров кожи[1], состав мочи). Кроме того, органы некоторых погибших животных подвергали патоморфологическому исследованию. Распределение никеля в органах, а также содержание его в моче и кале определяли методом эмиссионного спектрального анализа.

Результаты, характеризующие выживаемость различных видов животных при многократном поступлении в кровь мелкодисперсного металлического никеля, приведены в табл. 2.

Таблица 2

Выживаемость животных при многократном внутривенном введении металлического никеля (размер частиц 0,19 μ)

Животные	Суммарная доза (в мг/кг)	Число подопытных животных	Из них выжило	Из них пало	Срок гибели (в сутках): 1-е	2-е	3-и	4-е	5-е	6-е	7-е	8-е	9-е	10-е	12-е	15-е	22-е и позже
Крысы	600	10	0	10	—	—	1	—	5	1	1	1	—	—	1	—	
Мыши	600	10	0	10	—	—	—	2	3	1	1	2	—	—	—	1	
Крысы	500	5	1	4	—	—	—	—	1	1	1	—	—	—	—	—	1
Мыши	500	10	1	9	—	—	—	2	2	—	1	1	—	2	—	1	—
Крысы	400	5	2	3	—	—	—	—	—	1	1	—	—	—	—	1	—
Мыши	400	10	2	8	—	—	—	2	1	1	—	1	—	1	—	—	2
Крысы	300	5	2	3	—	—	—	—	2	1	—	—	—	—	—	—	—
Мыши	300	10	10	0	—	—	—	—	—	—	—	—	—	—	—	—	—
Кролики	250	5	0	5	—	—	—	—		—	—	—	—	—	—	—	—
Крысы	200	5	2	3	2	2	—	—	—	1	—	1	1	—	—	—	—
Мыши	200	10	8	2	—	—	—	—	—	—	—	—	—	1	—	—	1
Кролики	150	5	1	4		—	—	—	3	—	—	—	1	—	—	—	—
Крысы	100	5	4	1	—	—	—	—	—	1	—	—	—	—	—	—	—
Мыши	100	10	9	1	—	—	—	—		—	—	—	—	—	—	—	1
Кролики	50	5	2	3	—	—	—	—	2	—	—	—	—	—	—	—	1
Крысы	50	5	4	1	—	—	—	—	—	1	—	—	—	—	—	—	—
Мыши	50	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Крысы	25	5	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Мыши	25	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Крысы	10	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[1] Проницаемость изучали по методу Богдановой в модификации Иваницкого.

Из табл. 2 видно, что минимально смертельная доза никеля для белых мышей при этом способе введения — 100 мг/кг, абсолютно смертельная — 600 мг/кг.

Для крыс минимально смертельная доза несколько меньше, чем для мышей, и составляет 50 мг/кг. Наиболее чувствительными к введению препарата оказались кролики, абсолютно смертельная доза для которых составляла 250 мг/кг.

При сравнении данных, полученных в опытах на белых мышах в результате многократного и однократного введения им металлического никеля, оказалось, что как минимальная, так и абсолютно смертельная дозы в обоих случаях близки. Различия не превышают 25—100%. Это позволяет нам сделать вывод, что препарат металлического никеля обладает кумулятивными свойствами в отличие от растворимых его солей, которые этими свойствами не обладают (Черас, Я. М. Грушко, В. А. Донсков, В. С. Колесник и др.).

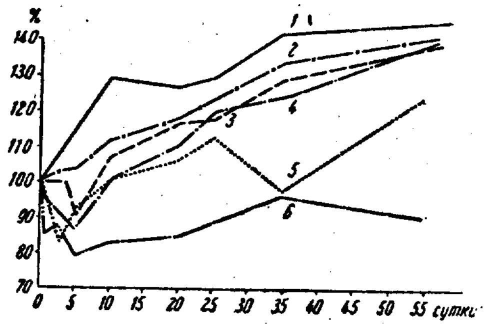

Рис. 1. Изменение веса крыс при многократном внутривенном введении металлического никеля (средние данные для каждой группы животных). *1* — контроль; *2*—25 мг/кг; *3*—50 мг/кг; *4*—100 мг/кг; *5*—200 мг/кг; *6*—500 мг/кг.

Клиническая картина поражения металлическим никелем у различных видов животных была однотипной. При введении больших, смертельных доз препарата (600—500 мг/кг для крыс и мышей, 250 мг/кг для кроликов) у животных появлялась вялость, жажда. У кроликов со 2—3-го дня опыта потребление воды увеличивалось с 50—100 до 150—200 мл в сутки; появлялось затрудненное дыхание, при этом в акте дыхания принимали участие межреберные мышцы; температура тела повышалась на 1—2°. Начиная с 4-х суток в моче определялся белок, сахар. Большинство подопытных животных погибало в первые 5—15 суток.

При меньших дозах препарата симптомы поражения были менее выраженными. Сроки гибели животных отодвигались на 22-е, 35-е, 70-е сутки и далее.

Для всех подопытных животных характерным являлось снижение веса тела в зависимости от введенной дозы препарата. Так, например, у крыс на 5-й день опыта отставание в весе при дозе 500 мг/кг достигало 30—40 г и составляло 20% от первоначального веса тела (рис. 1); при 200 и 50 мг/кг — соответственно 17 и 10%.

Исследование капиллярной проницаемости кожи показало, что у подопытных животных по мере увеличения срока после начала затравки скорость рассасывания флуоресцеинового пятна постепенно увеличивалась.

На 10—30-е сутки опыта она была максимальной и достигала 80—100% в час при 30—40% в час у контрольных животных. Повышение проницаемости было тем более выраженным и наступало тем быстрее, чем бо́льшую дозу никеля получали животные.

В периферической крови содержание эритроцитов и гемоглобина существенно не изменялось. Количество лейкоцитов вначале также оставалось постоянным. Однако на 6—15-е сутки у некоторых кроликов наблюдался нейтрофильный лейкоцитоз до 15—20 тысяч лейкоцитов в 1 мл крови.

При патоморфологическом исследовании у животных, погибших на 3—8-е сутки после введения никеля, в трахее и крупных бронхах обнаруживалась гиперемия[1]. Паренхима легкого была неоднородной по окраске и по консистенции: плотные сероватые очаги чередовались с участками розово-красного цвета.

При гистологическом исследовании отмечался резкий периваскулярный отек, гиперплазия перибронхиальной лимфоидной ткани, выявлялась катарально-десквамативная пневмония. В межальвеолярных перегородках и по ходу сосудов легких обнаруживались глыбки темно-бурого пигмента (рис. 2). Кроме того, наблюдались выраженное рас-

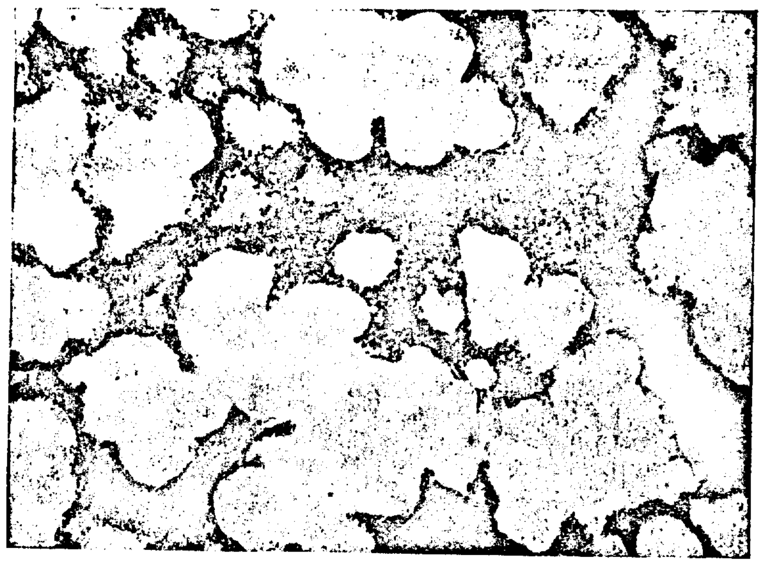

Рис. 2. Наличие никеля в кровеносных сосудах легкого у крысы, погибшей на 5-й день после ежедневного внутривенного введения препарата в общей дозе 300 мг/кг.

ширение капилляров и кровоизлияния в сердце, головном мозгу, полнокровие и очаговое набухание эпителия извитых канальцев и геморрагии в строме почек. Клетки ретикуло-эндотелиальной системы в органах, богатых ретикулярной тканью (печень, селезенка), находились в состоянии набухания и содержали мелкие зернышки темно-бурого пигмента.

У животных, погибших на 22—35-е сутки опыта, при гистологическом исследовании, кроме острых эксудативных изменений в легких, имелись и симптомы продуктивного воспаления в виде гиперплазии бронхиального эпителия и утолщения межальвеолярных перегородок (рис. 3).

Если животное выживало, то с 30—40-х суток от начала опыта общее состояние у него улучшалось, постепенно уменьшались признаки интоксикации. Однако полного восстановления исходного состояния не наступало. В поздние сроки выявлено раннее облысение, развитие подкожных абсцессов.

В нашу задачу не входило проведение специальных исследований с целью выяснения клинической картины отравления растворимыми соединениями никеля. Однако литературные данные говорят о том, что интоксикация этими соединениями протекает остро и характеризуется

[1] Гистологическое исследование препаратов проводилось при консультации старшего научного сотрудника А. П. Новиковой, которой выражаем нашу благодарность.

спазмами, рвотой, поносом, слюнотечением, отчетливым поражением центров, регулирующих дыхание и сердечную деятельность. В наших опытах при отравлении металлическим никелем указанных симптомов не отмечалось; наблюдалось преимущественно развитие поражений органов дыхания, сопровождавшихся нарушениями в других внутренних органах.

По-видимому, основное значение в механизме действия металлического никеля приобретает повреждающее действие мельчайшими части-

Рис. 3. Легкие кролика, погибшего на 22-й день опыта после внутривенного введения металлического никеля в общей дозе 50 мг/кг. Расширение капилляров, очаговый отек, начало формирования гранулем вокруг отложения никеля.

цами этого элемента, которые, попадая в различные органы и ткани, вызывают сначала местные нарушения — гиперемию, отек и другие признаки воспаления, что затем приводит к развитию общих расстройств организма. Естественно, местное действие проявляется сильнее в областях депонирования никеля. Возникал вопрос, не связано ли преимущественное поражение легких с максимальным раздражением этого органа в результате накопления здесь данного вещества.

Спектроскопическое изучение распределения никеля в организме подтвердило правильность этого предположения. В табл. 3 представлены данные, характеризующие содержание никеля в органах и тканях при внутривенном введении различных доз этого препарата.

Из табл. 3 видно, что максимальное содержание никеля отмечалось в легких в первые 5 суток с начала применения препарата и составляло около 15—20% от введенного количества никеля[1]. В почках, печени, селезенке, сердце, головном мозгу никель определялся от 0,1 до 0,5% от введенной дозы. На 6—7-е сутки содержание никеля в легких уменьшалось в 5—6 раз, что свидетельствовало о выраженной десорбционной способности этого органа. В других органах такого снижения не наблюдалось. У некоторых животных в эти сроки содержание никеля

[1] Вес легких у крыс в среднем 1,5 г, у кроликов — 20 г.

Таблица 3

Содержание никеля в органах в миллиграммах на 100 г ткани при внутривенном введении мелкодисперсного металлического никеля

Продолжительность жизни животных после отравки (в сутках)	Общая доза при отравлении (в мг/кг)	Легкие	Почки	Печень	Селезенка	Сердце	Головной мозг	Тонкие кишки	Толстые кишки	Мышцы	Желудок	Кость
				Крысы								
3	600	730	6	4,2	1,2	2,6	0,6	0,6	0,6	0,6	0,9	0,6
5	600	690	6	4,8	1,5	1,2	0,3	0,3	0,6	0,3	0,3	0,3
5	600	438	10,2	2,7	3,3	2,04	2,4	0,3	0,3	0,3	0,6	0,3
5	600	1000	4,8	3,6	9,6	8,4	6	1,8	0,6	0,6	3,0	0,6
5	600	500	3,9	2,1	2,4	4,0	6	1,8	0,6	0,6	3,0	0,6
5	600	510	8,1	7,2	1,2	1,8	0,9	0,3	0,3	0,6	1,2	0,6
5	500	420	4,5	5,4	4,8	3,6	0,6	0,6	0,6	0,6	0,6	0,6
5	300	281	2,79	2,8	0,6	0,3	3,3	0,3	0,3	0,6	1,2	0,3
5	300	249	4,5	1,5	0,6	2,49	0,6	0,6	0,6	0,6	0,8	0,6
6	300	81	4,8	2,4	4,4	0,6	0,6	0,9	0,6	0,6	0,6	0,6
6	600	156	11,4	3,6	5,7	2,7	0,6	0,6	0,6	0,6	0,9	0,6
7	600	161	5,1	1,5	0,9	0,9	0,6	0,6	0,9	0,9	1,5	0,9
7	500	110	1,8	1,74	0,6	3,9	0,6	0,6	1,2	0,3	1,2	0,3
8	600	27,9	1,2	4,2	0,6	0,3	0,3	0,6	0,6	0,6	0,6	0,6
12	600	15,6	0,9	0,9	0,9	0.6	0,6	0,6	0,6	0,6	1,2	0,6
15	400			Не обнаружен								
25	500			»	»							
				Кролики								
2	250	243	27	4,5	0,9	1,62	0,9	0,9	0,9	0,9	0,9	0,9
5	150	156	6,6	4,5	2,7	1,8	0,6	0,9	1,5	0,6	1,5	0,6
5	50	19,8	Следы	Следы	Следы	Следы	Следы	Следы	0,8	Следы	Следы	Следы
22	50	1,5							0,8			

в почках даже несколько возрастало. Однако во всех случаях легкие оставались наиболее богатыми данным веществом.

Выведение никеля из организма начиналось на 2—3-й день отравления и максимум приходился на 7-е сутки опыта; затем интенсивность этого процесса резко снижалась и через 9—10 суток никеля в выделениях практически не обнаруживалось. Выведение с калом превышало выведение с мочой в несколько раз.

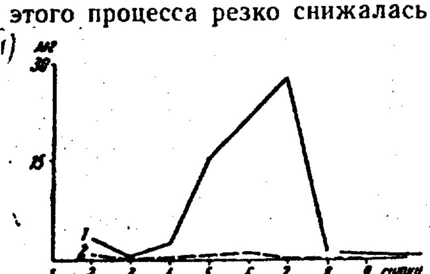

Рис. 4. Кривые выведения никеля с калом (*1*) и мочой (*2*) у крысы, получившей внутривенно 500 мг/кг металлического никеля.

На рис. 4 приведена характерная кривая выделения никеля из организма у белой крысы при внутривенной инъекции препарата в дозе 500 мг/кг.

Для выяснения вопроса о характере задержки исследуемого вещества тканью легкого были поставлены специальные опыты. Мы хотели выяснить, что имеет преимущественное значение — сорбция мелкодисперсного металлического никеля очень развитой поверхностью легкого или тропность легочной ткани к никелю. С этой целью было изучено распределение никеля в органах и тканях при введении его растворимых азотнокислой, сернокислой и углекислой солей (ионная форма Ni). В первые 3 суток никель накапливался в почках, печени, легких, селезенке, сердце. Однако уже к 6-м суткам в результате перераспределения его больше всего обнаруживалось в легких. Эти наблюдения дали нам основание сделать предположение о существовании некоторой тропности ткани легкого к никелю. Об этом говорят также и исследования органов и тканей кролика, не подвергавшегося отравлению никелем. Содержание этого микроэлемента в легких составляло ~ 0,016 мг на 100 г ткани. В других органах обнаружить никель при помощи нашего метода не удалось.

Представлялось интересным установить, при всех ли путях поступления мелкодисперсного металлического никеля поведение его в организме остается постоянным. С этой целью была поставлена третья серия опытов с введением данного препарата через рот в дозах от 50 до 1200 мг/кг. Под наблюдением находилось 70 белых мышей и 55 крыс. Условия эксперимента были те же, что и во второй серии.

При введении через рот испытуемый препарат в дозах до 100 мг/кг не оказывал существенного влияния на организм; 200—400 мг/кг вызывали вялость, снижение веса тела, температура тела сохранялась постоянной. Доза 500 мг/кг оказалась минимально смертельной для обоих видов животных, абсолютно смертельная доза составляла 1200 мг/кг. Средними смертельными дозами, рассчитанными по методу Беренса, являлись соответственно для мышей и крыс 940 и 780 мг/кг. Гибель животных при введении смертельных доз препарата наступала в большинстве случаев на 3—5-е сутки.

При вскрытии у животных желудок и кишечник были несколько гиперемированы, вздуты, заполнены пищевыми массами, окрашенными в черный цвет. Сосуды брыжейки были расширены. Макроскопических изменений в других органах не обнаруживалось.

В этой серии опытов распределение никеля в организме у животных отличалось от такового при внутривенном введении данного препарата.

Таблица 4

Содержание никеля в органах в миллиграммах на 100 г ткани у крыс при введении его через рот в дозе 750 мг/кг

Продолжительность жизни после затравки (в сутках)	Легкие	Почки	Печень	Селезенка	Сердце	Мозг	Мышцы	Кости
1	0,08	0,75	0,1	0,158	Следы	Следы	Следы	Следы
1	0,089	0,83	0,115	Следы	»	»	»	»
3	2,4	6,3	4,2	2,3	»	»	»	»
3	1,46	3,1	1,42	0,85	»	»	»	»
6	3,2	1,27	Следы	Следы	»	»	»	»
6	0,34	0,244	0,12	0,085	»	»	»	»
11	0,16	Следы	Следы	0,05	»	»	»	»
11	0,21	0,186	0,12	0,125	»	»	»	»

Из табл. 4 видно, что наибольшее количество никеля в первые 3 суток после введения наблюдалось в почках, печени, легких, селезенке, сердце и составляло около 0,5% от введенной дозы препарата. На 6-е сутки наряду с уменьшением абсолютных количеств никеля в органах и тканях наблюдалось также и его перераспределение. К этому сроку наиболее насыщенными никелем оказались легкие.

Основная масса никеля не всасывалась и выводилась с калом.

Заключение

Проведенные исследования показали, что мелкодисперсный металлический никель при многократном введении в организм оказывает выраженное токсическое действие. Особенно сильно это действие проявляется при внутривенной инъекции препарата. В этом случае минимально токсические и минимально смертельные дозы для крыс и мышей составляли соответственно 10 и 50 мг/кг, 25 и 100 мг/кг.

Более чувствительными к никелю из обследованных нами животных оказались кролики, наименее чувствительными — мыши.

Большинство подопытных животных, получавших смертельные дозы никеля, погибали на 5—15-е сутки. Причиной смерти являлась пневмония, сопровождавшаяся дистрофией паренхиматозных органов.

При внутривенном способе введения основное количество никеля обнаруживалось в легочной ткани. Можно предполагать, что это и является этиологическим фактором в развитии пневмонии.

Однако в общей картине интоксикации может иметь значение и поступление никеля в другие органы, о чем свидетельствуют, в частности, наблюдавшиеся изменения в печени, почках, сердце и других органах.

Интересно отметить, что избирательное накопление никеля в легких обнаруживалось нами несколько ранее и в опытах на кроликах при внутритрахеальном вдувании мелкодисперсного металлического никеля (эти животные также погибали от пневмонии)[1]. Мы объясняли этот факт барьерной функцией легочной ткани. Между тем известно, что при незначительных размерах отдельных частиц (меньше 1 μ) пыль, смешиваясь с воздухом и образуя аэрозоль, попадает через легкие непосредственно в кровь.

Проведенные исследования позволяют предположить, что при внутритрахеальном введении может иметь место и вторичное накопление в легких никеля, занесенного с кровью.

При попадании через рот металлический никель оказывает менее выраженное токсическое действие на организм, вызывая местные изменения в желудочно-кишечном тракте (гиперемия); от дозы 500—1200 мг/кг животные погибают. Исследования показали, что при этом способе введения основная масса никеля транзитом выводится с калом. В связи с этим в этих опытах процентное содержание исследуемого вещества в органах было значительно меньшим, чем при инъекции в вену, и составляло около 0,5% от введенной дозы.

Распределение никеля при введении через рот в первые дни опыта отличалось от распределения его при внутривенной инъекции. Наибольшие количества никеля накапливались в почках, печени, легких. Однако к 6-му дню опыта наблюдалось перераспределение с относительным увеличением содержания никеля в легких. Такое же распределение происходило и при отравлении растворимыми солями никеля. Это свидетельствует о том, что при попадании внутрь металлический никель частично растворяется в содержимом желудочно-кишечного тракта и всасывается в виде растворимых солей.

Выводы

1. Металлический никель с размером частиц 0,19 μ при многократном внутривенном введении оказывает выраженное токсическое действие на кроликов, крыс и белых мышей. Диапазон между минимально токсической и абсолютно смертельной дозами для крыс и мышей колеблется в пределах от 10 до 600 мг/кг. Абсолютно смертельная доза для кроликов — 250 мг/кг.

2. Токсическое действие металлического никеля на организм при введении через рот менее выражено. Минимально и абсолютно смертельные дозы для крыс и мышей — соответственно 500 и 1200 мг/кг.

3. Отмечена тропность никеля к легочной ткани.

При внутривенном введении металлического никеля содержание этого элемента в легких в течение всего периода исследования является максимальным, что может способствовать развитию пневмонии.

[1] Полученные результаты будут изложены в специальном сообщении.

При введении через рот распределение всосавшейся части никеля
аналогично распределению его растворимых солей. В первые 3 су-
ток он накапливается в почках, печени, легких, селезенке. На 6-е сут-
ки опыта в результате перераспределения наибольшее количество ни-
келя обнаруживается в легочной ткани.

4. Основное количество попавшего в организм никеля выделяется
в первые 7 суток.

Выведение никеля с калом превышает выведение его с мочой. При
внутривенной инъекции данного элемента эта разница составляет
400—600%.

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Поступила в редакцию 10/XI 1959 г

Journal of Biol. Chem.
178:315(1949)

THE EFFECT OF CATIONS ON THE DECARBOXYLATION OF OXALACETIC ACID*

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(Received for publication, September 27, 1948)

In connection with studies on the β-keto acid carboxylases of plants (1, 2), the effects of metal ions on the decarboxylation of oxalacetic acid, in the presence and absence of enzyme, were studied. In confirmation of Krebs (3) the decarboxylation was found to be accelerated by a variety of polyvalent cations. It was also observed that the enzyme oxalacetic carboxylase from parsley roots, which is nearly inactive in the absence of metal ions, is activated by a variety of divalent cations.

Methods

The experimental procedures used in studying keto acid decarboxylation are described in the preceding paper (2). Evolution of carbon dioxide from oxalacetic acid was measured in Warburg manometers, with a reaction mixture buffered at pH 5 to avoid retention. Since in most cases the decarboxylation reactions follow first order kinetics with respect to oxalacetic acid, the rates are conveniently expressed as first order reaction rate constants.

Results

Effect of Cations on Non-Enzymatic Decarboxylation of Oxalacetate—All the polyvalent cations tested, with the exception of Ba^{++}, accelerate the decarboxylation of oxalacetate. Monovalent cations were not studied, but Krebs (3) found them to be ineffective. In agreement with Krebs, it was noted that the effect of the cation is independent of the nature of the anion added with it, provided the salt is ionized. Except in the case of Fe^{+++} and Al^{+++}, which are described later, the decarboxylation reactions follow first order kinetics with respect to oxalacetic acid. When Cu^{++} is present in concentrations of 0.01 M or greater, the first order constants tend to decrease somewhat with time, and the average has been taken.

In Fig. 1 the first order rate constants are plotted against the logarithms

* This work was supported by grants from the John and Mary R. Markle Foundation and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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of the molarities of the cations. The curves for the various ions are similar in shape, but the concentration giving a maximum effect and the magnitude of the effect vary with different cations. Cu^{++} and La^{+++} give maxima at concentrations of 0.001 M. The activity maximum shown for Pb^{++} may

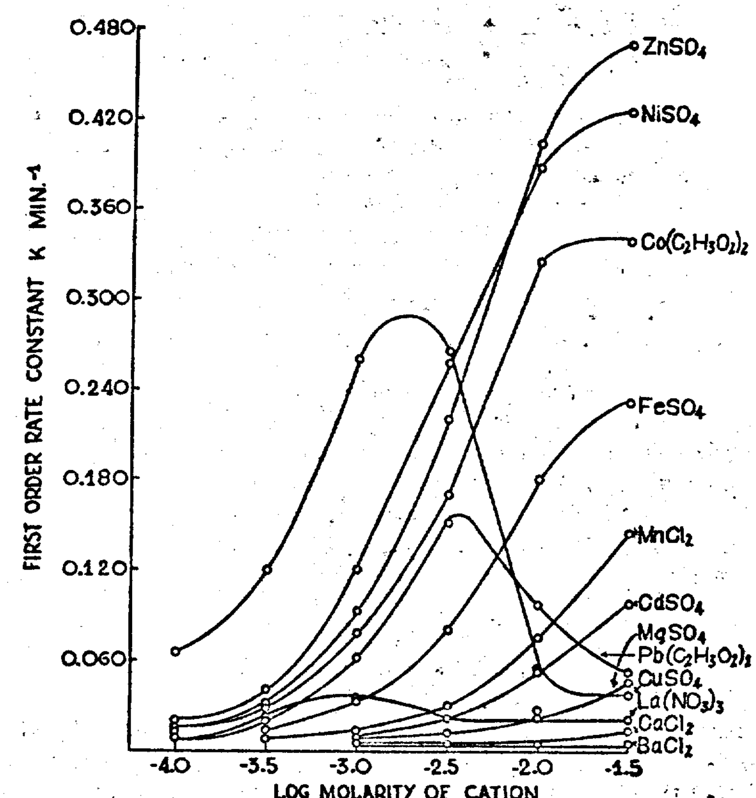

FIG. 1. Effect of various cations on the non-enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, 1 mg. of oxalacetic acid (equivalent to 160 μl. of CO_2), and metal salts in the concentrations indicated, in a total volume of 2.0 ml. Temperature 30°. In the absence of added polyvalent cation, the first order rate constant was 0.006 min.$^{-1}$.

not be similar in nature, because in samples containing Pb^{++} at concentrations of 0.01 M or greater a precipitate (perhaps lead oxalacetate) appears on tipping in the oxalacetic acid. Other ions, such as Zn^{++}, Ni^{++}, Co^{++}, and Fe^{++}, tend to approach maxima at the highest concentrations studied.

It was necessary to employ anaerobic conditions in testing the action of Fe^{++} ions. When air was used as the gas phase, a yellow color (probably due to formation of basic ferric acetate) appeared at the moment when the oxalacetic acid was tipped in. Subsequently oxygen was taken up at the same time that carbon dioxide was liberated, and the kinetics of the reaction were complex. Under anaerobic conditions (nitrogen as the gas phase,

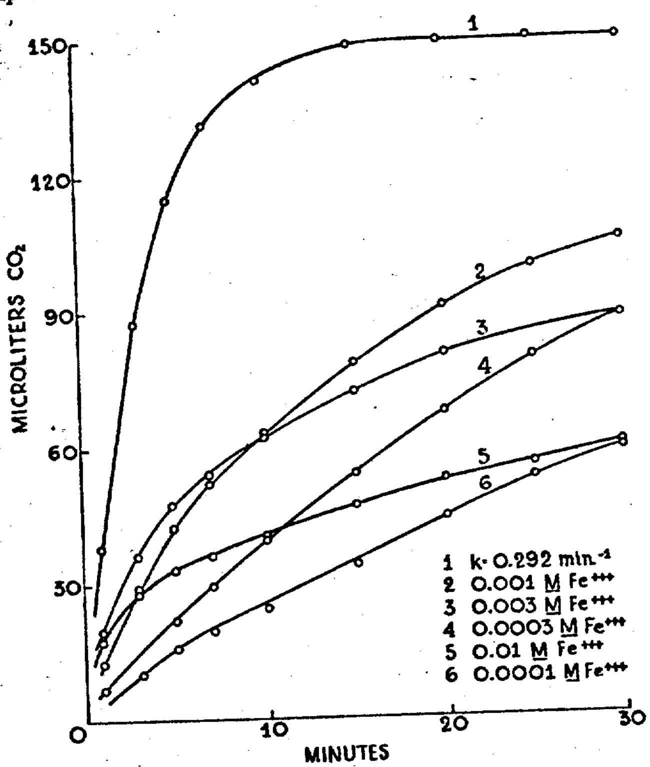

FIG. 2. Effect of Fe^{+++} on the non-enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, oxalacetic acid equivalent to 150 µl. of CO_2, and $FeNH_4(SO_4)_2$ in the concentrations indicated, in a volume of 2.0 ml. Temperature 30°; gas phase N_2. Curve 1 was calculated for a first order rate constant of 0.292 min.$^{-1}$, corresponding to the maximum rate with Cu^{++}.

yellow phosphorus in the center well), no yellow color appeared on tipping in oxalacetic acid, and the decarboxylation followed first order kinetics.

Fe^{+++} and Al^{+++} gave results different from those obtained with the other ions, and these effects are represented in Figs. 2 and 3. Decarboxylation of oxalacetate in the presence of these ions follows first order kinetics only at low cation concentrations (below 0.001 M). At higher concentrations the rates fall off more rapidly than expected for first order reactions, particularly in the case of Fe^{+++}. Both ions give maximum rates of decarboxylation at a concentration of about 0.001 M, and the maximum rates are lower than those observed with the most effective divalent cations, such as Zn^{++} and Ni^{++}. The experiments with Fe^{+++} were performed under anaerobic conditions.

Effect of Cations on Enzymatic Decarboxylation of Oxalacetate—A preparation of oxalacetic carboxylase from parsley roots, made as described in the

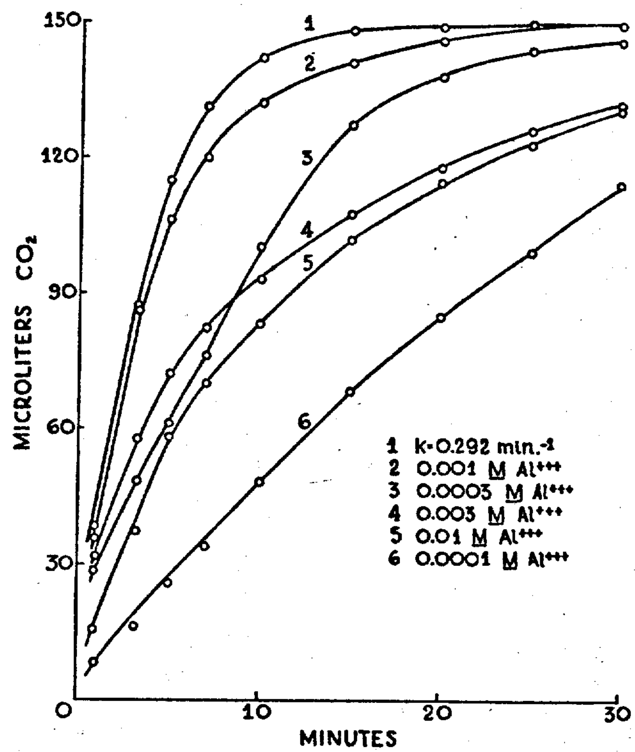

FIG. 3. Effect of Al^{+++} on the non-enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, oxalacetic acid equivalent to 150 µl. of CO_2, and $Al_2(SO_4)_3$ in the concentrations indicated, in a volume of 2.0 ml. Temperature 30°. Curve 1 was calculated for a first order rate constant of 0.292 $min.^{-1}$.

preceding paper, was used in studying the effect of cations on the enzymatic decarboxylation of oxalacetate. In the absence of added divalent cations, decarboxylation of oxalacetate by the enzyme is very slow. All the divalent cations studied activate the enzyme; *i.e.*, the rate of decarboxylation of oxalacetate in the presence of enzyme plus cation is greater than in the presence of cation alone, of enzyme alone, or of cation plus heat-inactivated enzyme. In all cases of activation, the reactions follow first order kinetics.

La^{+++} ions do not activate the enzyme, and Fe^{+++} and Al^{+++} ions give complex results, which are described later.

In Fig. 4 the relative activity of the parsley root enzyme in the presence of different cations is plotted against the logarithm of the cation concentration. The various ions give curves of similar shape which differ from

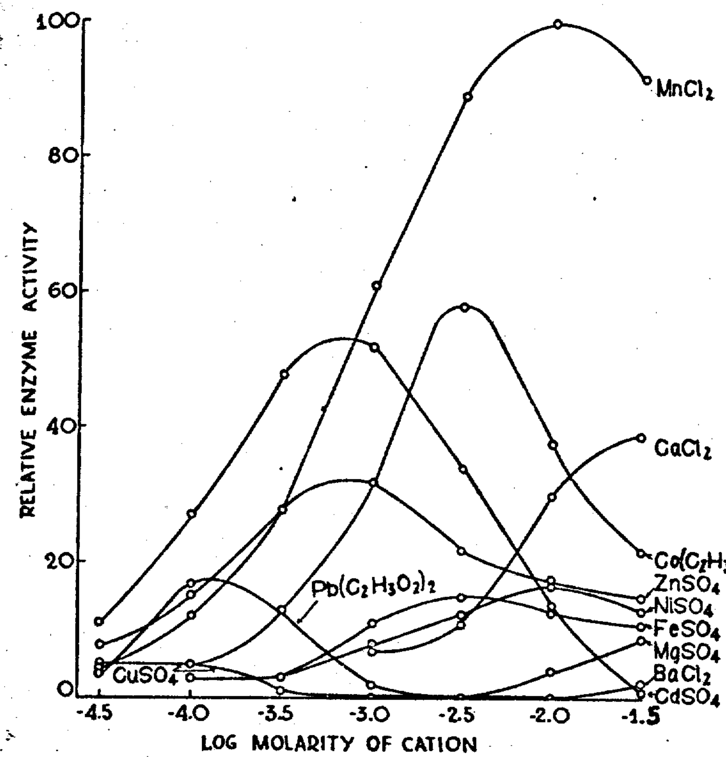

Fig. 4. Effect of various cations on the enzymatic decarboxylation of oxalacetate Samples contained 0.1 M acetate, pH 5.0, 1 mg. of oxalacetic acid, 15 mg. of lyophilized parsley root enzyme, and various ions in the concentrations indicated, in a volume of 3.0 ml. Temperature 30°. The rate with 0.01 M Mn^{++} is taken as 100. The relative rate in the absence of added divalent cations was 1.

each other in the position and height of the maximum. All of the ions except Mg^{++} and Ba^{++} show maximum effects within the range of concentrations studied. The maximum for Ca^{++} actually lies just at the highest concentration, 0.03 M; higher levels give lower enzyme activities. Of the ions tested, Mn^{++} is most effective in activating the parsley root enzyme.

In most cases the first order rate constants for the decarboxylation of oxalacetate in the presence of cation plus heat-inactivated enzyme are the same as in the presence of an equal concentration of cation alone. This indicates that the inactivated protein does not firmly bind these ions. However, the heat-inactivated protein reduces the rates obtained with Co^{++} and Ni^{++} at 0.03 M concentrations and with Zn^{++} at concentrations of 0.003 M and above. Decarboxylation of oxalacetate in the presence of heated

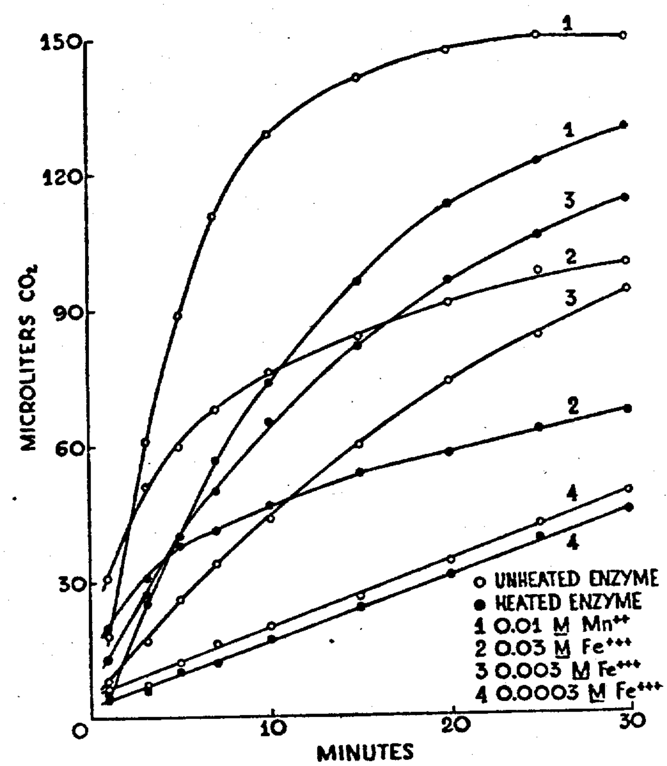

FIG. 5. Effect of Fe^{+++} on the enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, oxalacetic acid equivalent to 150 µl. of CO_2, 15 mg. of lyophilized parsley root enzyme, and $MnCl_2$ or $FeNH_4(SO_4)_2$ in the concentrations indicated, in a total volume of 2.0 ml. Temperature 30°.

enzyme and La^{+++} or Pb^{++} occurs at the same rate as that observed in the absence of added metal ions; the inactive protein abolishes the catalytic activity of these cations, although Pb^{++} activates the unheated enzyme.

Fe^{+++} and Al^{+++} gave anomalous results, which are represented in Figs. 5 and 6. Decarboxylation of oxalacetate in the presence of these ions does not follow first order kinetics. At cation concentrations of 0.0001 and 0.0003 M the rates in the unheated samples are the same as or slightly greater than in the heated samples; both rates are considerably lower than those observed with the ions in the absence of any protein (compare with Figs. 2 and 3). At cation concentrations of 0.001 and 0.003 M the rates in the heated samples are equal to or greater than those in the unheated samples; both rates are somewhat lower than those observed in the absence

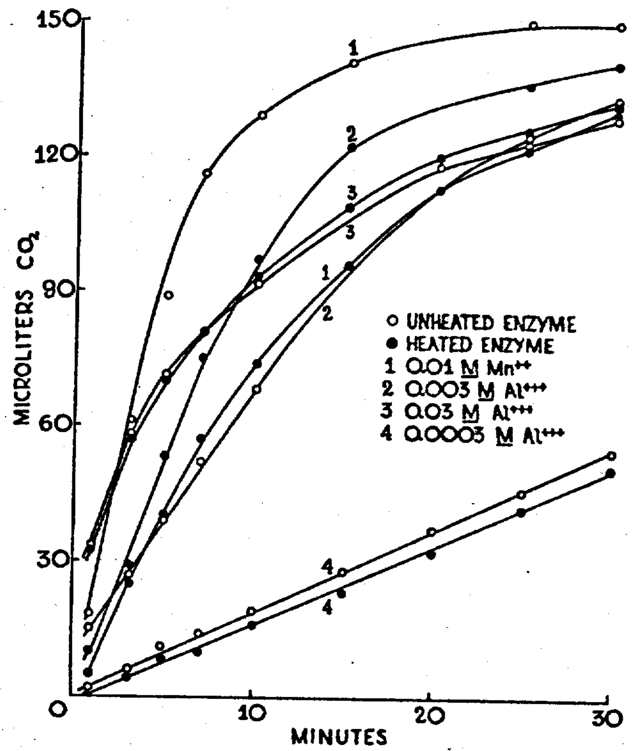

FIG. 6. Effect of Al^{+++} on the enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, oxalacetic acid equivalent to 150 μl. of CO_2, 15 mg. of lyophilized parsley root enzyme, and $MnCl_2$ or $Al_2(SO_4)_3$ in the concentrations indicated, in a total volume of 2.0 ml. Temperature 30°.

of protein. At cation concentrations of 0.01 and 0.03 M, the rates in the unheated samples are the same as or greater than in the heated samples; both rates are greater than those found in the absence of protein. An explanation for these results is not apparent. When Fe^{+++} was present in high concentrations, some tendency to form deposits of basic ferric acetate on protein particles was noted. In any case, it seems unlikely that either Fe^{+++} or Al^{+++} activates the parsley root carboxylase significantly.

The activity of the carboxylase with mixtures of two different cations, each in suboptimum concentration, was studied. In the presence of heated enzyme the effects produced by Zn^{++} and Cd^{++} are partially additive. The following first order rate constants were observed: 0.012 min.$^{-1}$ with 0.001 M $CdSO_4$, 0.030 min.$^{-1}$ with 0.0003 M $ZnSO_4$, and 0.037 min.$^{-1}$ with both. However, these cations compete in activating the unheated enzyme, so that intermediate activity is observed with both present. The relative enzyme activities (activity with 0.01 M $MnCl_2$ taken as 100) were 39 with 0.001 M $CdSO_4$, 23 with 0.0003 M $ZnSO_4$, and 35 with both.

Experiments were carried out in which the acetate buffer was replaced by benzoate, phthalate, oxalate, succinate, tartrate, or citrate of the same pH and molar concentration. 0.001 M $MnCl_2$ was present, and the rate of decarboxylation of oxalacetate was measured with and without enzyme. All of the buffers except benzoate reduce the rate of non-enzymatic decarboxylation below the level observed with 0.001 M Mn^{++} in acetate, and added enzyme is completely inactive. In the case of benzoate, the non-enzymatic rate is the same as with 0.001 M Mn^{++} in acetate, but added enzyme is inactive. Probably phthalate, oxalate, succinate, tartrate, and citrate decrease the decarboxylation rate by forming complexes with Mn^{++}, while benzoate more specifically inhibits the enzyme.

DISCUSSION

Krampitz and Werkman (4) first described the acceleration of the non-enzymatic decarboxylation of oxalacetate by metal ions for the particular case of Mg^{++}, and Krebs (3) investigated this effect of cations in greater detail. The results of his studies and of the experiments reported in the present paper may be summarized as follows: Many polyvalent cations accelerate the decarboxylation of oxalacetate. Of those tested, Zn^{++} and Ni^{++} are most effective. Anions and monovalent cations do not influence the reaction. This action of cations is general for the decarboxylation of β-keto dicarboxylic acids, such as oxalacetate, acetonedicarboxylate, and oxalosuccinate (5). Non-enzymatic decarboxylation of α- or β-keto monocarboxylic acids is not affected by metal ions.

Enzymes which catalyze the decarboxylation of oxalacetate have been found in bacteria (4, 6), animal tissues (7), and plants (1). All these enzymes require metal ions for full activity, and Mn^{++} has nearly always been employed. However, complete studies on the cation specificity of oxalacetic carboxylases from various sources have not been made. The carboxylase from parsley roots is activated by a considerable number of divalent positive ions, including Cu^{++}, Pb^{++}, Ba^{++}, Mg^{++}, Fe^{++}, Ni^{++}, Zn^{++}, Ca^{++}, Cd^{++}, Co^{++}, and Mn^{++}; Mn^{++} is most effective. There is no obvious correlation between the activities of the ions in the presence and absence of enzyme, but it appears that maximum activation of the enzyme is achieved at lower concentrations than are required for maximum rates of decarboxylation in the absence of enzyme. A similar carboxylase prepared from the red radish is active in the presence of Pb^{++}, Ni^{++}, Zn^{++}, Mg^{++}, Cd^{++}, Co^{++}, and Mn^{++}; Mn^{++} again gives the most rapid rates.[1] Oxalacetic acid carboxylase from pigeon liver is activated by Mn^{++} (7) and less effectively by Co^{++}.[2] The enzyme from *Micrococcus lysodeikticus* functions with Mg^{++} or Mn^{++} (4, 8) and that from *Escherichia coli* with Mn^{++} (6).

The concentrations of divalent cations in intact plant tissues are probably not sufficiently high to permit maximum activity of oxalacetic carboxylase unless the ions are localized at the site of enzyme action. For example, the Mn^{++} content of parsley and parsnip roots is only about 0.075 mM per 1000 gm. of fresh weight.[3] A number of the cations which activate the plant carboxylases are essential nutrients for plants; for example, Cu^{++}, Mg^{++}, Fe^{++}, Zn^{++}, Ca^{++}, and Mn^{++} (10). These ions may function in part as cofactors for oxalacetic carboxylase or other enzymes of wide distribution and possible great importance in plant metabolism.

Kornberg, Ochoa, and Mehler (8) have recently presented evidence that the effect of cations such as Al^{+++} and Mn^{++} on oxalacetate decarboxylation is due to formation of an unstable cation-oxalacetate complex, which decomposes to give pyruvate, carbon dioxide, and free cation. The carboxylase protein appears to accelerate the formation or breakdown of the complex. A number of cations seem capable of forming such complexes with oxalacetate; the affinity between metal ions and oxalacetate and the rate of decomposition of the complex vary with the different metal ions. The ability of enzymes to accelerate the formation or breakdown of certain of these complexes introduces a new element of specificity into the effects, since enzymes from different sources may vary in their activity with different ions.

SUMMARY

Decarboxylation of oxalacetic acid in the presence of polyvalent cations follows first order kinetics. The rates of decarboxylation with fourteen different ions over a range of concentrations are reported. The enzyme oxalacetic carboxylase from parsley roots is activated by a number of divalent cations, of which Mn^{++} is most effective. The relative enzyme activities with different concentrations of these metal ions are given.

[1] These experiments were performed by Miss Miriam C. Gollub of this department.

[2] Personal communication from Dr. Birgit Vennesland.

[3] The analyses were kindly carried out by Dr. Ernest Kun, using a procedure which he has recently described (9).

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A GENERAL METHOD FOR THE SYNTHESIS OF α,γ-DIAMINO ACIDS*

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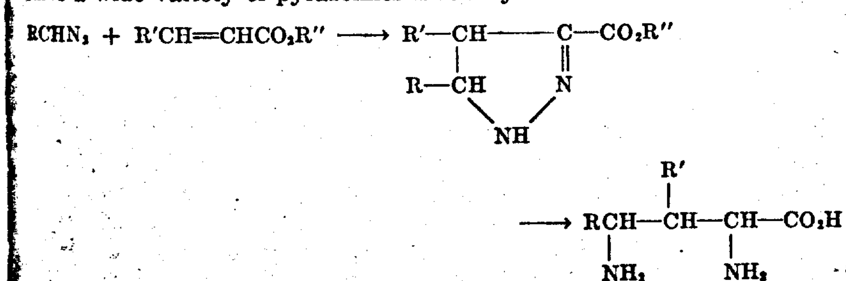
(Received for publication, October 28, 1948)

Carter and his associates (1, 2) have described the degradation of streptamine to α,γ-diamino-β-hydroxyglutaric acid. In order to compare this product with a compound obtained synthetically, a study of the preparation of α,γ-diamino acids was undertaken. Of particular interest was α,γ-diaminoglutaric acid, which might be expected to result from further degradation of α,γ-diamino-β-hydroxyglutaric acid. Another substance prepared in the course of this work, α,γ-diaminobutyric acid, is of current interest as a result of the discovery of its presence in the antibiotic substances polymyxin and aerosporin.

A survey of the literature revealed that no general method was available for the synthesis of α,γ-diamino acids and that very few compounds of this type were known. A number of investigators (3–7) have described the preparation of α,γ-diaminobutryic acid (L and DL forms). However, the methods employed were tedious, gave poor yields, and were not generally applicable.

In considering possible methods of synthesis of α,γ-diamino acids, the reduction of pyrazoline-3-carboxylic esters appeared promising, especially since a wide variety of pyrazolines is readily available.



* The authors wish to express their appreciation to the Abbott Laboratories, Eli Lilly and Company, Parke, Davis and Company, and The Upjohn Company for a generous grant in support of this work. Part of the material presented in this paper was taken from the thesis submitted by F. R. Van Abeele to the Graduate College of the University of Illinois in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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Can. J. Plant Sci. 49(3) 375-7 (1969)

NICKEL RESIDUES IN APPLE FRUIT AND FOLIAGE FOLLOWING A FOLIAR SPRAY OF NICKEL CHLORIDE[1]

Nickel salts have displayed fungicidal activity against plant pathogens (2, 5), and the absorption and movement of nickel in plants has been reported (6). The present research was designed to determine whether nickel, like mercury (4) and cadmium (3), accumulates in apple fruit and foliage following a single cover spray of nickel chloride. If nickel accumulates it could indicate the magnitude of the residues that may be expected from fungicides containing nickel.

Mature Cortland trees were sprayed to run-off with an aqueous solution of nickel chloride containing 37 ppm nickel when the apples were approximately 1 cm in diameter. A single tree was sprayed on June 25, 1964, and another on June 29, 1965. In 1967, three trees were sprayed on July 5. Fruit and foliage samples for analysis were collected immediately after the spray had dried and at approximately 3-week intervals throughout the growing season. At each sampling, 10 apples and 50 leaves were collected at random from each tree. Foliage samples were not taken in 1965. Sample preparation and digestion were carried out as previously described (3). Two determinations were done on each of the single samples taken in 1964 and 1967, and on the duplicate samples taken in 1965.

The analytical method of Alexander *et al.* (1), with the substitution of toluene (3) for chloroform and isoamyl alcohol, was employed to determine the nickel content of apple fruit and foliage digests. The optical density of the final extract was measured in a 5-cm cell at 385 mμ on a Beckman B spectophotometer. Recovery of nickel added to control samples of fruit and foliage at the levels found in this study was greater than 90%.

Initial nickel residues on foliage were 25 $\mu g/100\ cm^2$ leaf area in 1964 and 20 $\mu g/100\ cm^2$ in 1967. These residues declined throughout the growing season, and in October they were about 15 $\mu g/100\ cm^2$ in 1964 and 9 $\mu g/100\ cm^2$ in 1967 (Fig. 1). Since some of this decrease was due to increased leaf area, the leaf residues were quite persistent. Considering a rainfall of about 28 cm in both years and the high water solubility of $NiCl_2 \cdot 6H_2O$ (254 g/100 ml), the persistence of nickel in the leaves suggests that the nickel ion was rendered insoluble by reaction with some components of the leaf. Only traces of nickel were found in apple fruit and foliage from trees not sprayed with nickel.

Nickel accumulated in apple fruit following a single spray of nickel chloride (Fig. 2). In 1964, 1965 and 1967, initial deposits were 67, 41 and 71 μg/10 apples and harvest residues were 125, 147 and 199 μg/10 apples, respectively. This accumulation is probably due to translocation from the foliage. The amounts of nickel accumulating in the fruit were similar to those of mercury and cadmium following comparable sprays containing mercury (4) and cadmium (3). The peel/pulp nickel content ratios at harvest averaged 0.49, 0.70 and 0.75 for 1964, 1965 and 1967, respectively.

[1]Contribution No. 1332.

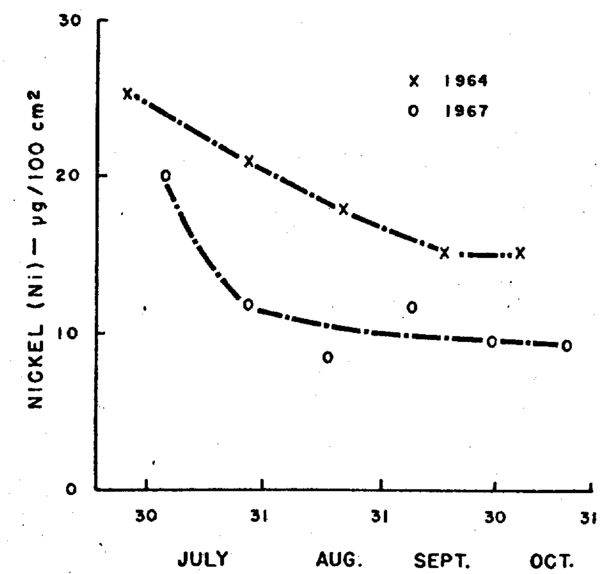

Fig. 1. Nickel residues in apple foliage following a single spray of nickel chloride.

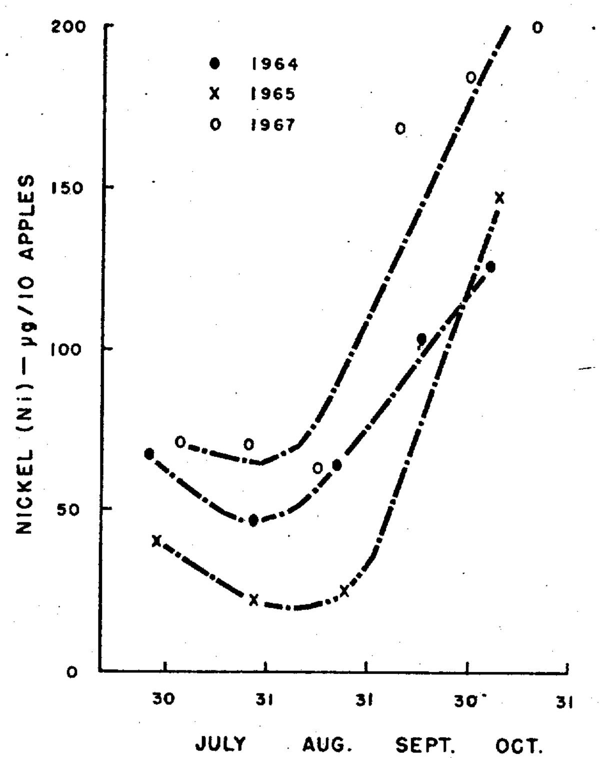

Fig. 2. Nickel residues in apple fruit following a single spray of nickel chloride.

Mercury from cover sprays of organic and inorganic mercurials, and nickel and cadmium from sprays of nickel and cadmium chlorides, accumulate in maturing apple fruit. The water solubility of the applied compounds does not appear to be an important factor in the movement of these three metallic elements. Considering the relatively large amounts of these elements deposited in the foliage, the amount translocated to the fruit is small. A possible explanation is that the metal is firmly bound in the leaf and that only small amounts are released and translocated. Further studies are needed to explain the nature of translocation to apple fruit.

The authors wish to thank K. G. Cairns for technical assistance.

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Received December 30, 1968.

Amer. J. Path.
46(6) 1027-1038(1965)

STUDIES OF NICKEL CARCINOGENESIS METASTASIZING PULMONARY TUMORS IN RATS INDUCED BY THE INHALATION OF NICKEL CARBONYL

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The high incidence of pulmonary cancer in nickel workers was first reported by Baader in 1937.[1] Since that time, the relation of nickel to pulmonary carcinogenesis has been the subject of a number of investigations.[2–21] In 1958, Doll[4] reported that 35.5 per cent of nickel workers in Wales died of cancer of the lung or upper respiratory tissues whereas the incidence among colliery workers was only 1.5 per cent. Within the past 2 years, Passey[15] tabulated 144 deaths from cancer of the lung in nickel workers and computed the average age at death in these workers to be 57.6 years. The average length of time that the affected workers were employed in nickel refineries was 27 years; the average time between the first exposure and death from lung cancer was 30.5 years. In Great Britain, cancer in the respiratory tract is a compensable disease for nickel workers.

The relationship between the inhalation of nickel and pulmonary cancer has been the subject of a number of investigations from our laboratory.[17–19] These studies have established that pulmonary cancer may be induced in rats exposed to a single heavy concentration of nickel carbonyl as well as in rats exposed to repeated inhalations of sublethal concentrations for a period of a year. It is noteworthy that cancers were not observed in these experimental animals until 2 or more years after the initial exposure.

In recent studies on pulmonary carcinogenesis,[16,20,22] nickel was found to be present in purified preparations of ribonucleic acid obtained from the livers and lungs of normal rats. In similar preparations from the livers and lungs of rats exposed to inhalations of nickel carbonyl, the amounts of nickel bound to RNA were found to be greater than those in

This study was supported in part by contracts of Jefferson Medical College with the Atomic Energy Commission and with the Rohm and Haas Company.

Accepted for publication, February 29, 1965.

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This is the seventeenth paper in a series of studies on nickel poisoning.

normal rats. Furthermore, specific changes in the physico-chemical properties of RNA were observed in purified preparations from the exposed animals.

Other studies from our laboratory have demonstrated the presence of nickel in tobacco smoke.[19] Computations of the amount of nickel inhaled annually by a heavy cigaret smoker indicate that this value is several times the amount that is carcinogenic for the rat;—an animal that is known to be unusually resistant to the development of pulmonary cancer.

The present studies covering a period of 3 years are a continuation of those previously undertaken on nickel carcinogenesis. Since "Dithiocarb" (N,N' sodium diethyldithiocarbamate-trihydrate) [23,24] has proved to be remarkably effective in the treatment of persons acutely poisoned by inhalations of nickel carbonyl, investigations were undertaken to ascertain whether or not the administration of "Dithiocarb" to animals subjected to lethal concentrations of nickel carbonyl would be protective against the induction or development of cancer in the respiratory tract. In addition, mortality data and weights of rats exposed to nickel carbonyl have been compared to those of unexposed animals under controlled conditions.

Material and Methods

Wistar strain, male white rats weighing between 200 and 250 gm were used in our experiments. The animals were kept under preliminary observation in cages adjoining the exposure chamber for a period of a month before being exposed to nickel carbonyl. Throughout the period of study, the rats were maintained on a diet of Purina® Rat Chow. They were observed daily and weighed weekly.

The design of the exposure chamber and the method of exposing rats to nickel carbonyl have been described in a previous publication from our laboratory.[25] In brief, nickel carbonyl is dissolved in a mixture of equal parts of absolute alcohol and ethyl ether and dispersed as a vapor into an air-stream flowing at a rate of 539 l per minute for a period of 30 minutes. During each period of exposure the concentration of nickel carbonyl in the chamber is determined by chemical analysis. The results of these analyses are compared to the calculated concentrations. In general, the chemical analyses yield values which are approximately 80 per cent of the calculated nominal concentrations.

In the present investigations, the rats were divided into 6 groups: 3 groups of control animals (III, IV and VI) and 3 groups of exposed animals (I, II and V). The disposition of the 6 groups was as follows:

Group I. Two hundred and eighty-five rats in this group inhaled nickel carbonyl in a concentration of 80 parts per million (0.6 mg per l) for a period of 30 minutes. Three weeks after exposure, 214 had died and only 71 of the exposed rats had survived and appeared healthy.

Group II. Sixty rats inhaled a single dose of nickel carbonyl for 30 minutes, in precisely the same manner as group I. In addition, however, each rat received "Dithiocarb" subcutaneously in a dosage of 50 mg per kg of body weight within 15 minutes after exposure. Three weeks after exposure all rats in this group had survived and appeared healthy.

Group III. Nineteen rats in this control group inhaled 5 ml of an alcohol-ether mixture (without nickel carbonyl) vaporized into the air-stream of the exposure chamber for 30 minutes. No fatalities occurred within 3 weeks after exposure and all animals appeared healthy.

Group IV. Nineteen rats in this control group inhaled 5 ml of an alcohol-ether mixture the same as group III, but in addition they received "Dithiocarb" subcutaneously in a dosage of 50 mg per kg of body weight immediately after removal from the exposure chamber. No fatalities occurred within 3 weeks after exposure and all animals were healthy.

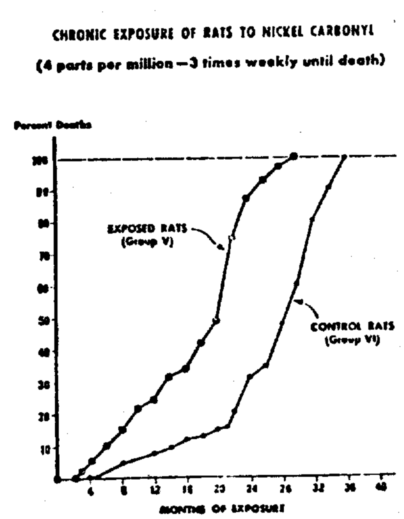
Group V. Sixty-four rats were exposed to inhalations of nickel carbonyl in a concentration of 4 parts per million (0.03 mg per l) for 30 minutes 3 times weekly for the remainder of their lives. All animals were alive after 3 weeks of exposures.

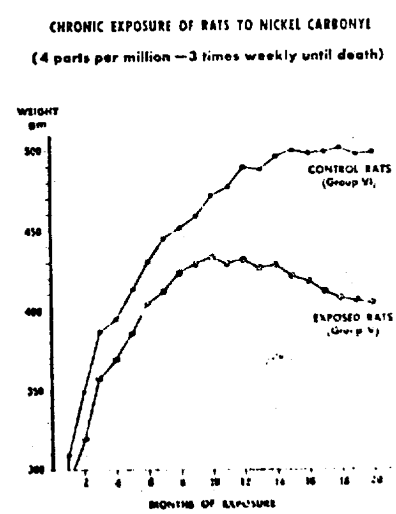
Group VI. This group of 32 rats served as controls for group V. These rats inhaled only alcohol-ether vapor (without nickel carbonyl) 3 times weekly for the remainder of their lives. No deaths occurred within the first 3 weeks after the initial exposure and all animals appeared healthy.

Necropsies were made on all animals that were sacrificed or found dead during the 3 year period of this study and representative tissues were removed and fixed for histologic studies. An attempt was made to avoid autolytic changes in the tissues by sacrificing animals that appeared moribund.

Results

Mortality and Weight Curves. In Text-figure 1 the mortality curve of rats (Group V) exposed chronically to the vapors of nickel carbonyl in a concentration of 4 parts per million (0.03 mg per l) for 30 minutes 3 times weekly until death is compared to the mortality curve of control rats (Group VI). At the end of 1 year, 25 per cent of the rats exposed to nickel carbonyl had died compared to 7 per cent of the controls. By the end of the second year, 88 per cent of the exposed rats had died compared to 30 per cent of the controls. The last rat of the exposed groups

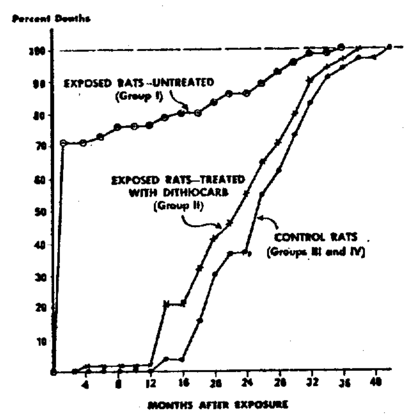




died 29 months after the initial exposure. The last control animal died 36 months after the beginning of the experiment.

In Text-figure 2 the mean weight curve of the rats chronically exposed to nickel carbonyl (Group V) is compared to the mean weight curve of the control animals (Group VI). It will be seen that the mean weight of the exposed rats was less than that of the control rats throughout the entire period of the study. At the end of 1 year of exposures to nickel carbonyl, the mean weight of the exposed rats was 430 gm. By comparison, the mean weight of the control animals was 490 gm. After 20 months of repeated exposures to nickel carbonyl, the mean weight of the surviving rats in this group was 405 gm as compared to 499 gm in the control animals (Group VI).

ACUTE SINGLE EXPOSURE TO NICKEL CARBONYL
(80 parts per million for 30 minutes)



In Text-figure 3 is portrayed the mortality curve of rats in groups I and II exposed to a single heavy dose of nickel carbonyl in a concentration of 80 parts per million for 30 minutes. This curve is compared to the mortality curve of control rats in groups III and IV. Within 3 weeks after exposure, 72 per cent of the rats in group I had died, whereas none of the rats in group II (similarly exposed but treated with "Dithiocarb") had died during this same period. The rat living the longest in group I died 34 months after exposure. One rat in the control group lived for 40 months after the experiment had been started. The mortality curve for the exposed rats in group II treated with "Dithiocarb" is similar to that of the combined curve of control groups III and IV.

Histologic Features of the Pulmonary Carcinomas Found in the Ex-

posed Rats. Of the rats in group V that were exposed chronically to nickel carbonyl, rat V-82 was sacrificed after 26 months of tri-weekly exposures. At death, the animal was emaciated, having lost 100 gm during the final week of life. At necropsy the left lung was found to be emphysematous and the right lung, though smaller, contained a neoplastic lesion. Both kidneys were enlarged and contained lesions considered to be tumors. Microscopically the main pulmonary tumor was an adenocarcinoma (Fig. 1). The glandular pattern in various areas of the tumor resembled bronchiolar structures, peribronchial glands or even hyperchromatic alveolar elements with some papillary irregularities. The neoplastic change, although characterized by a major tumor, includes multicentric lesions ranging from early neoplastic transitions in alveolar epithelium (Fig. 2) to distinct masses that could be either satellite metastases from the main tumor or additional primary tumors developing from some of the multicentric foci. Metastatic adenocarcinoma was found in the regional mediastinal lymph nodes, and in the myocardium (Fig. 3). The metastases were differentiated enough to be readily recognizable as compatible with origin in the primary pulmonary lesion.

Rat I-462 died 24 months after a single exposure to nickel carbonyl. The lungs were grossly congested and in the right lung, there was a tumor nodule, approximately 1.0 cm in its greatest diameter. The liver was the site of nodules ranging from a few mm to slightly less than a cm in diameter. Unfortunately, the tissues were generally affected by autolysis. The lung tumor, however, could be readily recognized histologically as a papillary adenocarcinoma with necrosis (Fig. 4). The renal and hepatic metastases (Fig. 5) were clearly recognizable as secondary to the papillary adenocarcinoma of the lung.

The third carcinoma of the lung encountered in our investigation occurred in rat II-479, an animal exposed only once to nickel carbonyl and subsequently treated with "Diothiocarb." Death occurred 26 months after exposure. The animal was emaciated and had gross evidence of tumor in the lungs and liver with definite enlargement of the spleen. Histologically, the lungs were the site of anaplastic carcinoma of an expansile type (Fig. 6) with multicentric sites or metastases. Similarly, anaplastic metastases were encountered around the hilum of the enlarged hyperplastic spleen (Fig. 7) as well as in the liver (Fig. 8). Extensive necrosis was present in the carcinoma.

The last rat to survive in control group VI died 33 months after the initial exposure. The histologic study in this rat VI-1 revealed a single well-circumscribed, but not encapsulated, nodule approximately 1 mm in diameter (Fig. 9). The lesion was a papillary adenomatous tumor and had some degree of nuclear hyperchromatism. In character this tumor was similar to 2 small papillary adenomas discovered in the lung of a test rat in an earlier study.[17] In that study it was pointed out that the lesions were similar to one reported by Horn and Stewart,[26] who classified it simply as a spontaneous pulmonary tumor in the rat, fundamentally similar to the papillary glandular pulmonary tumor of the mouse. We consider such tumors to be akin to those classified by Mostofi and Larsen[27] as "adenomatoid alveolar cell tumors" found in the lungs of certain strains of mice. Most investigators avoid any designation of them as malignant and we, too, accept the more conservative view.

Additional Pathologic Findings. Previous experience[17,18] indicated that protracted follow-up observation for at least 2 years was necessary for the discovery of pulmonary tumors resulting from exposure of rats to nickel carbonyl. It was also discovered that chronic inhalation of a toxic agent frequently resulted in a relatively high mortality owing to inflammatory disease and other conditions which might not be related to neoplasia.

Pulmonary lesions other than neoplastic types were practically all inflammatory and consisted mainly of pneumonitis, pneumonia, bronchitis, bronchiectasis, bronchial abcesses and reactive fibrosis. These lesions occurred commonly and were frequently lethal. Such changes were encountered in the lungs of older rats and could be extensive.

In the present studies the animals in the control groups as well as those exposed to the inhalations of nickel carbonyl were beset with inflammatory lung lesions. Therefore, there was no attempt to assign an etiologic role to nickel carbonyl in explaining the inflammatory reactions or reparative fibrosis, though the possibility of such a role is recognized and has been suggested by earlier studies on acute nickel carbonyl poisoning.[28,29]

In Table I are listed the rats that developed malignant tumors or related conditions. It will be seen that a variety of types of tumors was encountered among our experimental animals. Most of these lesions were not considered related to nickel carbonyl exposure since rats in the control groups developed similar lesions.

In the 3 control groups that were never exposed to nickel carbonyl, 44 rats were alive at the end of the 2 year period of observation and of these, approximately one-third (15) developed malignant tumors. In the 3 test groups chronically or acutely exposed to nickel carbonyl, 80 rats were alive at the end of the 2 year period of observation and of these approximately one-half (39) developed malignant tumors. Malignant lymphoma was the most common neoplasm found in both control

TABLE I

NEOPLASMS FOUND IN CONTROL RATS AND RATS EXPOSED TO NICKEL CARBONYL

Group	Type of exposure	No. of rats with specific types of tumors	
I	$Ni(CO)_4$, single exposure of 80 ppm for 30 minutes (of 285 rats exposed, 71 survived)	Malignant lymphomas *	9
		Myelogenous leukemia	1
		Fibrosarcoma (subcutaneous)	1
		Osteosarcoma and metastases	1
		Leydig cell carcinoma of the testis	1
		Squamous carcinoma of skin	1
		Papillary adenocarcinoma of lung with metastases	1
		Subcutaneous fibrosarcoma, malignant pheochromoblastoma (included in the lymphoma group because of its dominant lesion)	1
II	$Ni(CO)_4$, single exposure of 80 ppm for 30 minutes followed by "Dithiocarb" intraperitoneally (of 60 rats exposed, all survived)	Malignant lymphomas *	8
		Fibrosarcoma (subcutaneous and widespread metastases)	4
		Carcinoma of the skin	2
		Anaplastic carcinoma of the lung with metastases	1
		Anaplastic malignant tumors of uncertain origin, probably sarcoma	2
III (control)	Alcohol-ether, single exposure to concentrations similar to group I (of 19 rats exposed, all survived)	Malignant lymphomas *	3
		Angiosarcoma with metastases	1
		Squamous carcinoma of skin	1
IV (control)	Alcohol-ether, single exposure in concentrations similar to group I and then followed by "Dithiocarb" intraperitoneally (of 19 rats exposed, all survived)	Malignant lymphomas *	4
		Fibrosarcoma (intra-abdominal)	1
		Adenocarcinoma of thyroid	1
V	$Ni(CO)_4$, chronic, multiple exposures of 4 ppm for 30 minutes 3 times weekly until death (of 64 rats exposed, all were alive 3 weeks after exposures)	Malignant lymphomas *	5
		Squamous carcinoma in salivary gland and an adrenal carcinoma	1
		Adenocarcinoma of the lung with metastases	1
VI (control)	Alcohol-ether, chronic, multiple exposures in concentrations similar to group V (of 32 rats exposed, all were alive 3 weeks after exposures)	Malignant lymphoma *	1
		Myelogenous leukemia	1
		Fibrosarcoma (intra abdominal)	1
		Adenocarcinoma of pancreas (probably ductal)	1

* Nearly all of the malignant lymphomas in all groups can be classified as histiocytic (reticulum cell) sarcomas and the most common major involvement was in the lungs or mediastinum.

and test animals. Other types of tumors included fibrosarcoma, myelogenous leukemia and squamous cell carcinoma of the skin.

Three rats exposed to nickel carbonyl developed pulmonary carcinoma with metastases. The incidence of three pulmonary carcinomas among the 80 rats that lived to or beyond the 2 year latent period after exposure to nickel carbonyl is considered highly significant. This significance is based not only on the fact that no pulmonary carcinomas had developed in the control rats with comparable longevity but also on a number of reports in the literature[29,30,31] dealing with the incidences of spontaneous tumors in rats. These reports indicate that the lungs in rats are peculiarly resistant to primary pulmonary carcinoma.

TABLE II

PULMONARY CANCER WITH METASTASES IN RATS EXPOSED TO NICKEL CARBONYL

Type of exposure	Concentration of $Ni(CO)_4$ in parts per million	Death of rats months after initial exposure	Type of tumor
3 times weekly for 1 year	4	24	Squamous cell carcinoma
3 times weekly for 1 year	4	24	Squamous cell carcinoma
3 times weekly for 26 months	4	26	Adenocarcinoma
Single exposure	35	27	Anaplastic carcinoma
Single exposure	80	24	Adenocarcinoma
Single exposure	80	26	Anaplastic carcinoma

In Table II are listed 6 rats that developed pulmonary carcinomas with metastases following exposure to nickel carbonyl in this study and in an earlier one.[17] These neoplasms occurred among 89 rats that lived 2 years or more after the initial exposure to nickel carbonyl. The lesions were classified as squamous cell carcinoma (2); adenocarcinoma (2); and anaplastic carcinoma (2). All of the lesions were found between 24 and 27 months after the initial exposure to nickel carbonyl. Three of the rats had been subjected to mild chronic exposures and 3 had been subjected to a heavy single exposure.

DISCUSSION

Pulmonary carcinoma occurs only rarely as a spontaneous lesion in rats. In our series of experiments over a period of 12 years, carcinoma of the lung had never been encountered in an untreated control rat. On the other hand, 6 rats exposed to nickel carbonyl were found to have pulmonary carcinomas with metastases. With the exception of Gillman, Gilbert and Spence,[32] many investigators have emphasized the rarity of spontaneous pulmonary carcinoma in experimental rats.[17,18,26,30,31,33] The spontaneous pulmonary tumors in a group of 327 rats reported by Gillman and co-workers were described as being similar histologically to the urethane-induced lesions observed in mice by Mostofi and Larsen.[27] Mostofi classified these lesions as "adenomatoid alveolar cell tumors in mice" and did not indicate that they were malignant. Gillman and co-workers,[32] however, suggested that the 3 tumors they found in the lungs were carcinomas, indistinguishable from the adenomatous tumors reported by Mostofi. We, too, have found occasional papillary adenomas in rat lungs but have considered such lesions to be benign. One such tumor was found in a control rat VI-1 which lived 33 months. In this tumor, the histologic sections did not suggest malignancy (Fig. 9) and no evidence of metastasis was found in this rat. Mostofi observed no metastases in his studies and pointed out that he would not refer to such urethane-induced lung tumors in mice or rats as carcinomas.[34] The incidence of tumors in rats reported in the studies of Gillman and co-workers is strikingly different from that in the series of 31,868 rats reported by Curtis, Bullock, and Dunning,[30] and from that in the series of 498 rats reported by Saxton, Sperling, Barnes and McCay[31] in which neither reported the finding of any carcinomas in the lungs. In view of these reports, the interpretations of Gillman and associates are open to uncertainty.

Owing to the histologic character of some of the multicentric lesions observed in the lungs in our rats we are led to suggest that small circumscribed lesions characterized by moderate hyperchromatism in nuclei and papillary infolding of alveolar walls may represent relatively early morphologic evidence of neoplastic change. Such changes could possibly eventuate in malignant neoplasm if carcinogenic influences continue to be active.

The question of a possible effect of nickel carbonyl on the incidence of other types of tumors seems far less certain. The most common type of neoplastic disease in all our groups of animals was malignant lymphoma, frequently a histiocytic or reticulum cell sarcoma. The anatomic sites most frequently involved were the peribronchial regions and the mediastinal lymphoid tissues. The occurrence of lymphomas among older rats has been previously noted. Crain[33] found a total of 41 malignant lymphomas among 786 rats of which 189 had 200 tumors. Approximately one-third of the tumors were malignant and these included the 41 lymphomas. Saxton, and co-workers,[31] reported lymphosarcomas of the lung among 234 tumors in rats. In our present series of 265 rats living more than 3 weeks after exposure, 30 developed lymphoma. A relationship to nickel exposure appears doubtful in view of the high spontaneous incidence of lymphoma in rats reported in the literature and found among our control animals.

Our present investigations involved a limited test of the protective action of N,N′ sodium diethyldithiocarbamate-trihydrate against the carcinogenic action of inhaled nickel carbonyl in rats. Although no positive protective action was apparent in these studies, it should be emphasized that "Dithiocarb" was not given prophylactically and therefore, the results are inconclusive.

In earlier studies,[17,19] it had been shown that carcinoma of the lung developed in rats that inhaled nickel carbonyl thrice weekly for one year in an estimated total dosage of 1930 μgm of nickel. The amount of nickel contained in the main stream smoke of a single cigaret was shown to be 0.37 μgm. The amount of nickel inhaled by the experimental rats is comparable to that contained in the main stream smoke of approximately 260 packs of cigarets.

Summary

Our studies indicate that inhaled nickel carbonyl is carcinogenic to the lungs of rats, a species generally considered to be peculiarly resistant to pulmonary cancer. In a combined series of studies, 6 rats exposed to nickel carbonyl developed pulmonary carcinoma with metastases. The lesions included the common types of pulmonary cancer, squamous cell carcinoma, adenocarcinoma and anaplastic carcinoma. All of the pulmonary lesions were found between 24 and 27 months after the initial exposure to nickel carbonyl.

The amount of nickel capable of inducing lung cancer in the rat is comparable to the amount of nickel inhaled by persons smoking less than 15 cigarets per day for a period of a year.

The mean weight of rats chronically exposed to nickel carbonyl was found to be consistently less than that of the control rats throughout the entire 3-year period of study.

After 2 years of chronic exposure to nickel carbonyl, the percentage of deaths in the exposed group of rats was approximately 3 times greater than in the control groups.

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LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Adenocarcinoma of lung, rat V-82 sacrificed after 28 months of tri-weekly exposures to nickel carbonyl. × 160.

FIG. 2. Hyperplastic folds of alveolar lining cells with hyperchromatic nuclei. These lesions, considered to be "early" neoplastic transitions, were found scattered through the same lung pictured in Figure 1. × 160.

FIG. 3. Metastatic adenocarcinoma invading heart muscle in rat V-82. There is more fibrotic stroma in this lesion than in the primary tumor shown in Figure 1 or in the other secondary tumors. × 160.

FIG. 4. Adenocarcinoma of lung in rat I-462 which died 24 months after a single exposure to nickel carbonyl. The tissue is moderately autolyzed but the papillary character of the neoplasm is easily recognized. × 160.

FIG. 5. Metastatic adenocarcinoma in liver secondary to the pulmonary lesion shown in Figure 4. × 160.


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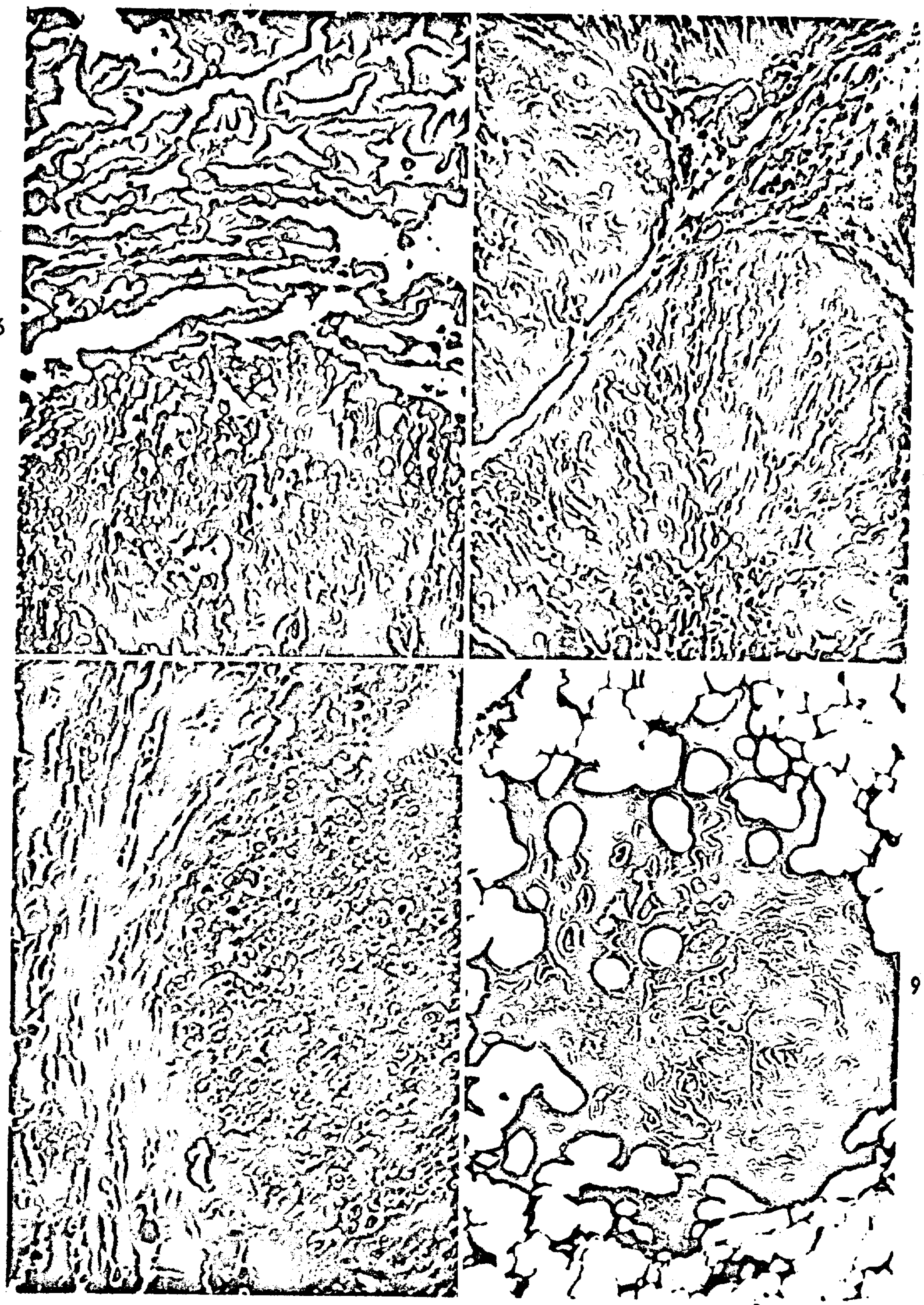

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FIG. 6. Relatively solid and apparently expansile anaplastic carcinoma in the lung of rat II-479 also exposed only once to nickel carbonyl. Death occurred 26 months after exposure. × 225.

FIG. 7. Section of a representative area in one of many secondary anaplastic tumor masses found in extrapulmonary regional sites in rat II-479. These masses exhibit the same expansile character as the primary tumor shown in Figure 6. × 225.

FIG. 8. Liver with partially necrotic anaplastic carcinoma secondary to that portrayed as primary pulmonary carcinoma in Figure 6. × 225.

FIG. 9. Well circumscribed papillary adenomatoid alveolar cell tumor in the lung of control rat VI-1. This is considered to be a benign lesion. × 52.5.

FIG. 10. Higher magnification of the central area in the tumor shown in Figure 9. The papillary and alveolar character of the benign lesion is evident at this magnification × 445.

The American Journal of Clinical Pathology
Vol. 35, No. 3, pp. 203–209
March, 1961

NICKEL POISONING

XI. Implication of Nickel as a Pulmonary Carcinogen in Tobacco Smoke

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For the past several years, we have been engaged in studying the toxic effects of inhalations of nickel and, in particular, the vapors of nickel carbonyl.[15, 16, 24, 27-29, 31, 32] Recently our interest has been focused on the carcinogenic effects of inhalations of nickel carbonyl upon the respiratory tract.[23, 25] The present paper is concerned with the implication of nickel as a carcinogen in tobacco smoke.

Whenever finely divided nickel or its compounds come in contact with carbon monoxide, nickel carbonyl is formed. Nickel carbonyl is widely used commercially and is one of the most toxic gases encountered in industrial operations. The 22nd American Conference of Governmental Hygienists in April 1960[1] placed the maximal atmospheric concentration for a working day at 1 part per billion (by comparison hydrogen cyanide was placed at 10 parts per million). Nickel carbonyl is unstable under atmospheric conditions and if inhaled, nickel is presumed to be deposited in highly active form on the respiratory mucosa. The high volatility of nickel carbonyl creates a special hazard of exposure by inhalation during handling.

The toxic effects from exposure to nickel carbonyl are both acute and chronic. After acute exposure severe symptoms may develop insidiously and death ensue. We suspect that acute nickel carbonyl poisoning may not be too uncommon and that the occurrence of acute nickel pneumonitis usually goes unrecognized.[24, 28]

Within recent years it has been shown that nickel workers have a high incidence of cancer of the respiratory tract.[3, 7, 17, 18] An historical resumé of published studies on the relation of nickel to cancer of the respiratory tract is presented in Table 1. Baader[2] in 1937 suggested a prevalence of pulmonary cancer among nickel workers. Although Campbell's studies[5] in 1943 were inconclusive, nevertheless, he suggested that the incidence of lung cancer in mice was increased after exposure to nickel dust. In 1949 the Ministry of Pensions and National Insurance of Great Britain officially designated lung cancer in workers exposed to "nickel produced by decomposition of a gaseous nickel compound" as an industrial disease.[8, 10] Løken[17] in 1950 reported 5 instances of lung cancer in nickel workers in Norway. Barnett[3] noted that, from 1923 to 1948 inclusive, 82 cases of cancer of the lung with 72 fatalities and 49 cases of cancer of the nose with 46 fatalities occurred among nickel workers in Great Britain. In 1957 Sunderman and co-workers[25] demonstrated that rats exposed to inhalations of nickel carbonyl developed extensive squamous metaplasia of the bronchial epithelium. Surviving rats were observed for pulmonary carcinogenesis. Morgan[18] analyzed the incidence of respiratory tract cancer in nickel workers in Wales and in 1958 reported 131 cases of lung cancer and 61 cases of nasal cancer. In 1958 Doll[8] reported a statistical study of the cause of death among workmen in Glamorganshire, Wales, in relation to their last employment. He found that between the years 1938 and 1956, 35.5 per cent of all of the deaths in nickel workers was caused by cancer of the lung and nose; by comparison only 1.5

Received, October 14, 1960; accepted for publication November 3.

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Presented at the meeting of the American Society of Clinical Pathologists, Chicago, Illinois, September 27, 1960.

This study was supported in part by contract of Jefferson Medical College with the Atomic Energy Commission.

TABLE 1

CANCER OF RESPIRATORY TRACT FROM EXPOSURE TO NICKEL

1937	*Baader* announced high incidence pulmonary cancer in nickel workers
1944	*Campbell* suggested that incidence of lung cancer in mice was increased after exposure to "nickel dust"
1949	*Ministry of Pensions*, Great Britain, officially designated cancer of respiratory tract in nickel workers as an industrial disease
1950	*Løken* reported 5 cases of lung cancer in nickel workers in Norway
1952	*Barnett* reported 82 cases of lung cancer and 49 of nasal cancer in nickel workers from 1923–1948
1957	*Sunderman et al.* demonstrated extensive squamous metaplasia of the bronchial epithelium in rats exposed to nickel carbonyl. Surviving rats were observed for pulmonary carcinogenesis
1958	*Morgan* reported 131 cases of lung cancer and 61 of nasal cancer in Welsh nickel workers
1958	*Doll* reported 35.5 per cent of all deaths among nickel workers in Glamorganshire to be due to cancer of the respiratory tract
1958	*Williams* reported a pathologic study of 5 cases of lung cancer in Welsh nickel workers
1958	*Hueper* found metaplastic and anaplastic changes in lungs of rats and guinea pigs exposed to nickel dust
1959	*Sunderman et al.* induced pulmonary cancer in rats by inhalations of nickel carbonyl

per cent of cancer of the lung and nose occurred in coal miners living in the same district during the same period. In 1958, Williams[33] published autopsy findings in 5 cases of lung cancer in nickel workers. Hueper[13] reported metaplastic and anaplastic changes in the lungs of rats and guinea pigs exposed to nickel dust. In 1959 Sunderman and associates[23] succeeded in inducing pulmonary cancers in rats by the inhalation of nickel carbonyl.

Our studies on the induction of pulmonary cancer in rats exposed to nickel carbonyl were facilitated by the use of an exposure chamber which we have previously described.[26] The pulmonary cancers were induced in our rats by 2 methods of exposure to nickel carbonyl: (1) a single heavy exposure to a concentration of 250 μg. of nickel carbonyl per liter of air for 30 min. and (2) multiple exposures to a concentration of 30 μg. of nickel carbonyl per liter of air for 30 min. 3 times weekly for 1 year. The estimated amounts of nickel inhaled which induce pulmonary cancers in rats are given in Table 2. It is estimated that the rats surviving a single heavy exposure inhaled 115 μg. of nickel and that the rats subjected to chronic exposure inhaled 1930 μg. of nickel during the year. It is noteworthy that the pulmonary cancers in our rats were not observed until 2 years or more after the initial exposure. It might be emphasized that the induction of pulmonary cancer in a laboratory rat is a severe challenge, inasmuch as spontaneous pulmonary neoplasms occur only rarely in this animal.[12, 20, 34]

Descriptions of the types of pulmonary cancer observed in rats exposed to the inhalations of nickel carbonyl are contained in a previous publication.[23] Long term studies of pulmonary carcinogenesis induced by repeated sublethal doses of nickel carbonyl are being continued in our laboratory and will be the subject of a subsequent report.

In view of our findings that the inhalation of minute amounts of nickel is carcinogenic for rats, a study was undertaken to evaluate nickel as a possible carcinogenic agent in

TABLE 2

CARCINOGENIC DOSAGES OF INHALED NICKEL CARBONYL FOR RATS*

	Inhaled $Ni(CO)_4$	Inhaled Ni
	μg.	μg.
Survivors from single heavy exposure (250 μg. $Ni(CO)_4$ per liter for 30 min.)	300	115
One year—chronic exposure† (30 μg. $Ni(CO)_4$ per liter for 30 min.; 3 times weekly)	6700	1930

* Ventillation-minute volume for rat = 0.040 l.; 30-min. volume = 1.2 l.

† $Ni(CO)_4$ inhaled per exposure = 36 μg.

smoking tobaccos. Nickel is a ubiquitous element present in trace amounts in practically all soils and plants. According to Bear[4] soils of New Jersey contain between 2 and 40 p.p.m. of nickel; plants from the same area contain nickel in concentrations of 0.5 to 2.5 p.p.m. on a dry-weight basis. Nickel has been reported to be present in trace amounts in tobacco by Kennaway and Lindsey[14] and in concentrations of 1.9 μg. per Gm. by Cogbill and Hobbs.[6]

Nickel has a great affinity for carbon monoxide. In the burning of tobacco, carbon monoxide is formed in amounts estimated to be from 2 to 7 per cent of the smoke.[19, 21, 30] Nickel or nickel compounds in a finely divided state will unite with carbon monoxide to form nickel carbonyl, even at environmental temperatures; however, maximal concentrations at normal pressure are obtained at 45 to 50 C., which is within the range of the temperature of the combustion gases from burning cigarettes.[9, 11] In a burning cigarette all of the reactants and the reaction conditions are present which are known to lead to the formation of nickel carbonyl. In view of these facts tobacco smoke obviously provides a ready means for transporting an active form of nickel into the respiratory system.

EXPERIMENTAL STUDIES

Measurements were undertaken in our laboratory to determine the amount of nickel in common brands of American cigarettes. Six different brands were analyzed quantitatively for nickel by a method developed previously in our laboratory.[15]* The average nickel content per cigarette for each brand is listed in Table 3. Brands "A" to "E" inclusive were non-filter cigarettes; brand "F" contained a filter. It will be seen that the amount of nickel in the brands tested varied from 1.59 to 3.07 μg. per cigarette and that the mean for the 5 non-filter brands was 1.99 μg. Brand "C," containing 1.85 μg., was closest to the mean and was selected for partitioning measurements of nickel in the ash and smoke, respectively.

* Method given in "Appendix B, Determination of Nickel in Biologic Materials" of cited reference.

TABLE 3

NICKEL IN 6 BRANDS OF CIGARETTES

Ni (μg.) per Cigarette					
A	B	C	D	E	F (Filter)
1.59	1.75	1.85	2.30	2.48	3.07
Mean = 1.99					

The apparatus which was assembled for the analysis of nickel in smoke is illustrated in Figure 1. The smoking chamber is cylindrical in shape to provide uniform mixing and is capable of accommodating 5 cigarettes. The smoke from burning cigarettes is drawn by intermittent vacuum through 4 Vigreux type absorption columns connected in series. The first 2 columns are filled with an alcoholic-iodine solution[15] for the extraction of nickel carbonyl and the last 2 columns are filled with dilute nitric acid (1:1) for the extraction of inorganic nickel. Four columns were required to obtain complete recovery of known mixtures of nickel carbonyl in air. In practice the apparatus was set to operate at a negative pressure and a flow rate which approximated the suction and volume of smoke inhaled by smokers tested in our laboratory. The smoke from a single cigarette was drawn through the apparatus at a flow rate of approximately 225 ml. per min. per cigarette operating under a negative pressure of 20 mm. of water during a burning period of 4½ min. It was observed that adherence to these operating conditions was best achieved by smoking 1 cigarette at a time.

In Table 4 are given our values for the nickel partition of cigarette "C." The values represent the mean of 15 duplicate measurements on 30 packs of cigarettes purchased from various sources. Each of the 30 analyses for total nickel was made on a pool of 5 cigarettes; each of the nickel analyses of ash and main-stream smoke was made on pools of 15 cigarettes. The amount of nickel contained in cigarette "C" which weighs approximately 1 Gm., was 1.85 μg. S.D. ± 0.22. Of this total amount of nickel, 71.4 per cent was recovered in the ash and butt (20 mm.) and 20.0 per cent was recovered

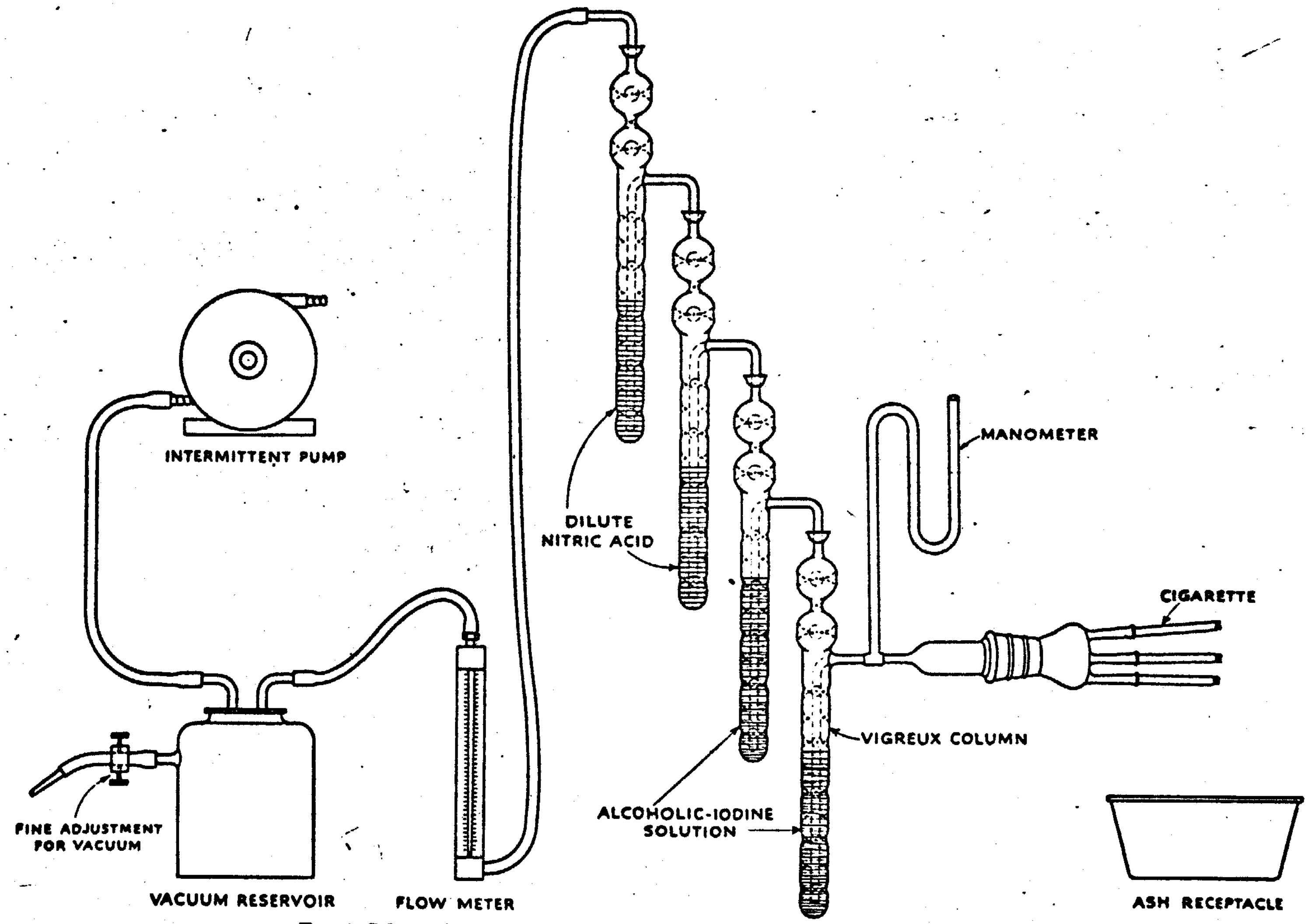

FIG. 1. Schematic diagram of apparatus used for collection of nickel in smoke

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in the main-stream smoke. By difference, 8.6 per cent was lost in the side-stream smoke.

The nickel partitions of brands "C" and "F" cigarettes, brand "P" cigar and brand "G" pipe tobacco are listed in Table 5. All of the values are expressed as μg. of nickel per Gm. of freshly opened tobaccos. The greatest amount of nickel per Gm. of tobacco was contained in brand "P" cigar (3.19 μg. per Gm.). It is noteworthy that the nickel contained in the main-stream smoke in various tobaccos was between 12.9 and 26.6 per cent of the total nickel. Filters from unsmoked cigarette "F" yielded no trace of nickel; filters from *smoked* cigarette "F" contained an average of 0.04 μg.

Comparisons have been made of the amount of nickel inhaled by our chronically exposed rats that developed pulmonary cancer and the amount of nickel that would be inhaled by a person smoking 2 packs of cigarettes daily, assuming that he inhaled all of the main-stream smoke. These values are given in Table 6. The average ventilation minute-volume for rats is reported to be 40 ml.[22] Therefore, during a 30-min. exposure to nickel carbonyl in a concentration of 30 μg. per l., the amount of nickel inhaled would be 12.4 μg. Our rats that developed cancer of the lung had been exposed 3 times weekly for a period of 1 year. Therefore, the total amount of nickel inhaled during the year may be estimated as 1930 μg. By comparison, a person inhaling the main-stream smoke from 2 packs of cigarettes a day for 1 year would inhale 5400 μg. of nickel. This estimate is 3 times the amount that was observed to be carcinogenic for laboratory rats. In our studies pulmonary cancers also developed in rats surviving an acute exposure of 115 μg. of inhaled nickel. The calculated nickel inhalations of 5400 μg. for heavy cigarette smokers is approximately 47 times this value.

TABLE 4
NICKEL PARTITION FOR CIGARETTE "C"

	Ni (μg.) per Cigarette	Per Cent of Total
Total nickel	1.85, S.D. ± 0.22	100.0
Ash + butt (20 mm.)	1.32, S.D. ± 0.23	71.4
Main-stream smoke	0.37, S.D. ± 0.16	$\boxed{20.0}$
Side-stream smoke (by difference)	0.16	8.6

TABLE 5
NICKEL PARTITION IN TOBACCOS

	Ni (μg.) per Gm. of Tobacco			
	Cigarette "C"	Cigarette "F"	Cigar "P"	Pipe "G"
Total nickel	1.84	3.07	3.19	2.72
Ash + butt	1.31	2.34	2.01	2.20
Main-stream smoke	0.37	0.58	0.85	0.34
Side-stream smoke (by difference)	0.16	0.11	0.33	0.18
Filter		0.04		
Ni in main-stream	20.0%	18.9%	26.6%	12.9%

TABLE 6
COMPARISONS OF NICKEL INHALATIONS

	Nickel Inhaled	
	μg.	μg.
Carcinogenic dose for rats: 1 year of chronic exposure (30 min.; 3 times weekly)	12.4 (per exposure)	1930 (per year)
Main-stream cigarette smoke (2 packs per day)	14.8 (per day)	5400 (per year)

DISCUSSION

In a recent study of a number of metallic constituents in cigarettes, Cogbill and Hobbs[6] reported cigarettes to contain 2 μg. of nickel. This value is in accord with our mean value of 1.99 μg. for 5 non-filter brands of cigarettes and with our value of 1.85 μg. (S.D. ± 0.22) for cigarette "C." Cogbill and Hobbs failed to detect nickel in condensates of main-stream cigarette smoke. Their condensates were obtained by passing smoke through a series of cold traps backed by flasks packed with glass wool. In the light of our analyses it would seem that Cogbill and Hobbs either failed to trap nickel by their cooling procedure or lost volatile nickel during the preparation of their samples. It is also possible that the nickel might have been adsorbed during entrainment. There are probably a number of factors that may inhibit the recovery of nickel vapor. For example, in earlier studies we encountered adsorption of nickel on plastic tubing. In our present investigations the tobacco smoke was passed directly through alcohol-iodine and nitric acid solutions. With this procedure no difficulty was encountered in recovering nickel from tobacco smoke.

In industrial operations elaborate detection systems have been devised and stringent regulations are enforced to protect workers from both acute and chronic exposures to vapors of nickel carbonyl. In an effort to approach the proposed average value for maximal atmospheric concentration of nickel carbonyl in air of 1 part per billion for a working day, the attitude has been adopted that any detectable exposure to nickel carbonyl is excessive and should be eliminated. Our present studies indicate that nickel in the main-stream cigarette smoke is present in concentrations of approximately 140 parts per billion. It is, therefore, logical that efforts should be made to eliminate nickel vapors from tobacco smoke.

SUMMARY

Measurements have been made of the nickel content in cigarettes and of the partition of nickel in the ash and butt, mainstream and side-stream smokes. Six common brands of American cigarettes contained from 1.59 to 3.07 μg. of nickel per cigarette. Twenty per cent of the total nickel was recovered from the main-stream smoke. Similar measurements were made on cigar and pipe tobaccos.

The carcinogenic effects of inhaled nickel upon the respiratory tract have been reviewed. It has been estimated that the amount of nickel inhaled in 1 year by a heavy cigarette smoker is approximately 3 times the amount of inhaled nickel which we have previously reported to be carcinogenic for the rat—an animal notably resistant to the induction of pulmonary cancer.

In the light of these investigations it is our considered opinion that attempts should be made to remove nickel from tobacco smoke.

SUMMARIO IN INTERLINGUA

Esseva effectuate mesurationes del contento de nickel in cigarrettas e del distribution de nickel inter cineres e resto e le fumo del currentes central e lateral. Sex sortas de cigarrettas american de uso commun contineva inter 1.59 e 3.07 μg. de nickel per cigarretta. Vinto pro cento del nickel total esseva trovate in le fumo del currente central. Simile mesurationes esseva effectuate in cigarros e tabaco de pipa.

Es revistate nostre cognoscentias del effecto carcinogene del inhalation de nickel super le vias respiratori. Esseva estimate que le quantitate de nickel inhalate in le curso de un anno per un forte fumator de cigarrettas es approximativemente 3 vices le quantitate de nickel le qual—secundo un previe reporto nostre—es carcinogene post inhalation per rattos, e rattos es notabilemente resistente contra le induction de cancere pulmonar.

In le lumine de iste investigationes il es nostre firme conviction que on debere effortiar se a eliminar nickel ab le fumo de tabaco.

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J. Am. Med. Assoc. 155:889-94 (1954)

NICKEL POISONING

II. STUDIES ON PATIENTS SUFFERING FROM ACUTE EXPOSURE TO VAPORS OF NICKEL CARBONYL

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Studies were recently reported by us [1] on the effects of acute and subacute exposure of experimental animals to the vapors of nickel carbonyl. In this paper are reported the results of clinical investigations on two groups of people exposed to this material: one group consisted of about 100 workmen exposed within a period of a few hours while engaged in the repair of a plant reactor contaminated with nickel carbonyl [2]; the other group consisted of five subjects exposed individually under varying working conditions and at different times and locations.

Nickel carbonyl was discovered by Mond, Langer, and Quincke [3] in 1890, and its extremely toxic nature was recognized shortly thereafter. In 1891 McKendrick and Snodgrass [4] concluded that a concentration of even less than 0.5% in air was dangerous. Later investigations have shown that this value is much too high and that the lethal concentration is about in the same range as that of hydrogen cyanide. The maximal allowable concentra-

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From the Division of Metabolic Research, Jefferson Medical College of Philadelphia, and Research Division, Rohm and Haas Company.

This study was supported in part by contract with Atomic Energy Commission.

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3. Mond, L.; Langer, C., and Quincke, F.: Action of Carbon Monoxide on Nickel, J. Chem. Soc. London 57:749-753, 1890.
4. McKendrick, J. G., and Snodgrass, W.: On Physiological Action of Carbon-Monoxide of Nickel, Brit. M. J. 1:1215-1217, 1891.

tion of nickel carbonyl was set at one part per million in air by the American Conference of Governmental and Industrial Hygienists in 1948.[5]

In our previous study on experimental animals [1] it was shown that $L.D._{50}$ values for 30 minutes' exposure for mice, rats, and cats are about 10, 35, and 270 parts per million by volume, respectively. Studies of the subacute toxicity in these animals indicated that the toxic effects were not cumulative and that tolerance could develop after exposure to sublethal concentrations. In addition, these studies indicated that the administration of dimercaprol (BAL) to rats exposed to nickel carbonyl increased their tolerance to this material by a factor of two.

SYMPTOMS AND PATHOLOGICAL CHANGES

The symptoms in humans suffering from acute exposure to nickel carbonyl have been described by Armit,[6] Amor,[7] Bayer,[8] Brandes,[9] Carmichael,[10] and others.[11] There is general agreement that the symptoms of acute poisoning are of two types, initial and delayed. Even with exposure sufficiently severe to cause death, the initial symptoms are usually mild and not specific, disappearing quickly on removal of the subject to uncontaminated air. Initial symptoms generally include frontal headache, dizziness, and sometimes nausea and vomiting. In the delayed reaction, tightness in the chest, cough, dyspnea, weakness, and gastrointestinal symptoms are frequently observed. According to Amor,[7] the delayed reactions are ushered in by the development of retrosternal pain. The respiratory rate appears to be a useful guide in predicting the severity of the exposure. In the severer cases, the respiratory rate is increased out of proportion to the pulse rate, so that a pulse-respiratory ratio of 3:1 or less during the initial stage suggests that the person may go into the delayed stage. A review of 354 cases by the Mond Nickel Company gives the following symptoms in the severe cases in their order of frequency: 56% of the patients complained of frontal headache, 42% giddiness, 33% tightness in chest, 28% nausea, 22% weakness of the limbs, 16% perspiring, 15% cough, 14% vomiting, 9% cold and clammy skin, and 9% shortness of breath.

There are relatively few descriptions in the literature of the pathological findings in cases of acute poisoning with nickel carbonyl. Amor,[7] Bayer,[8] and Brandes [9] have reported autopsy studies in cases of human exposure; Armit,[6] Barnes and Denz,[12] and Kincaid, Strong, and Sunderman [1] have studied the changes occurring in the organs of experimental animals exposed to lethal concentrations of the vapor.

The main pathological changes occur in the lungs, liver, and brain. The cause of death is regarded as being primarily due to loss of aerating tissue in the lungs. Throughout the lungs, areas of hemorrhage, atelectasis, and necrosis are frequently found. The pulmonary epithelium may become severely damaged. Bayer [8] reports that in his cases the alveoli were filled with a filamentous, coagulated material containing few erythrocytes and practically no leukocytes. Infiltration of connective tissue into the lungs is also reported by him.

Barnes and Denz [12] made a careful study of the tissues of rats and rabbits after the inhalation of nickel carbonyl in lethal or near-lethal concentrations. Their studies left little doubt that the primary cause of death was due to severe damage to pulmonary tissue. In their animals extensive pulmonary edema developed within a couple of hours after exposure. This was followed by capillary congestion, interstitial edema, and dilatation of the lymphatic vessels. After the fourth day, the pulmonary edema regressed and the alveoli became invaded with leukocytes, macrophages, and fibroblasts. It should be especially noted that Barnes and Denz [12] observed that the normal thin alveolar wall in the lungs of their animals was replaced by a remarkably thick, cellular structure of young connective tissue, which appeared to reach a maximum infiltration about three months after exposure and "then slowly resolved." They noted that rats examined a year or more after exposure did not show the presence of excessive fibrous tissue or other evidence of damage to the lung.

Kincaid, Strong, and Sunderman [1] report the deposition of brownish-black noniron staining pigment throughout the alveolar walls of the lungs in their experimental animals that died a few hours after exposure. Armit [6] indicated that probably the most outstanding pathological change observed in his exposed animals was degeneration of the endothelial cells of capillary vessels. Barnes and Denz suggest that the pulmonary damage may be caused by abnormal enzymatic activity in the capillary endothelium.

Except congestion, no unusual changes have been reported in the liver after human exposure; on the other hand, extensive degenerative changes with necrosis about the central veins are found histologically in liver sections from exposed animals. Kincaid and his associates [1] found brownish-black pigment deposited in the hepatic cells of rats. This pigment was similar to that also found in the lungs. Several cases have been reported in human beings in which extensive damage occurred to the brain. Dilated vessels and hemorrhages have been reported as well as the suffusion of the meninges with blood.[13] Armit [6] observed petechial hemorrhages in the adrenal glands of his exposed animals. These hemorrhages were found most frequently in the medullary portions of the glands.

Within recent years the high incidence of carcinoma of the respiratory passages among workers of long service in nickel refineries has come to be recognized.[14] Ac-

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13. Amor,[7] Brandes.[9]
14. (a) Hueper, W. C.: Occupational Tumors and Allied Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1942, pp. 417-418. (b) Løken, A. C.: Lung Cancer in Nickel Workers, Tidsskr. norske lægefor. 70: 376-378, 1950.

cording to Hueper,[14a] the apparent carcinogenic property of nickel carbonyl was first observed by Baader in 1924 and the first report that an excessive number of cases of carcinoma of the lungs and nasal passages developed among nickel workers was made by Grenfell in 1932. An analysis of death certificates issued in South Wales between 1907 and 1934 indicated that 34% of the cases of cancer of the respiratory organs in South Wales occurred in nickel workers.[14a] Barnett[15] noted that, from 1923 to 1948 inclusive, 49 cases of cancer of the nose with 46 fatalities and 82 cases of cancer of the lungs with 72 fatalities were reported from a nickel works in England. The average number of years worked before the development of cancer was 23 for patients with nose cases and 25 for patients with lung cases.

CLINICAL OBSERVATIONS

Within recent months an opportunity was afforded to study a group of persons exposed to nickel carbonyl during the repair of a reactor in an oxo plant of one of the large oil refineries in Texas.[2] The reactor was surrounded by a stall that hindered the dispersal of fumes. Only persons who entered this stall became ill. The length of exposure within the stall varied from about 12 hours to a few minutes. A total of about 100 men were exposed, 31 required hospitalization, and 2 of these died.

In this accident, it appears clear that the patients were exposed essentially to the vapors of nickel carbonyl and not carbon monoxide. These cases, therefore, differ from many of those described in the early literature in which the toxic agent was presumably a mixture of nickel carbonyl and carbon monoxide. Evidence to support the diagnosis of nickel carbonyl poisoning without simultaneous carbon monoxide exposure are the following: 1. Air samples taken on three occasions during the period of exposure were all found to be free of carbon monoxide. 2. A careful study of the operating conditions that existed at the time of the accident showed that the carbon monoxide that did not form nickel carbonyl had been vented into a stack before the workmen entered the contaminated area. 3. Clinically, the men did not reveal any discoloration of the skin and mucous membranes suggestive of carbon monoxide poisonings.

Only a synopsis of the clinical observations will be given at this time.[16] The symptoms observed in the hospitalized men were characteristically of the initial and delayed types. The most prominent initial symptom after exposure was dizziness accompanied by a severe, throbbing headache. In more than one-half of the hospitalized cases, the men became nauseated after exposure and many of them vomited. Soon after exposure, most of the men experienced a sense of constriction in the chest with concomitant shortness of breath and dry, hacking, unproductive cough. In only one patient was a history of productive cough elicited.

In a number of the patients, the initial symptoms merged gradually into the more severe, delayed reactions; in others, the transition from one stage to another was more abrupt. Usually, the severe stage was begun by a paroxysm of coughing. The onset of the severe symptoms varied from 10 hours to 8 days after exposure.

A universal complaint of the critically ill men was that of extreme weakness and ready fatigue. Most of the men were too weak to turn over in bed. Some stated that they felt too exhausted to breathe. In a high percentage of cases, respirations could not be sustained without pressure oxygen therapy. Breathing was rapid, shallow, and panting in type. Respiratory rates above 60 per minute were observed in certain patients. Several of the critically ill patients had symptoms referable to the central nervous system. The two men who died had convulsions terminally; others became irrational.

None of the hospitalized men had any discoloration of the skin or mucous membranes initially, although most of them had cyanosis with the onset of the delayed symptoms. It might be mentioned that examination of the blood of several of the men spectrophotometrically five days after exposure revealed an absence of carboxyhemoglobin or any other abnormal pigments.

Diarrhea and abdominal distention occurred in several of the patients two or three days after exposure. This might suggest that nickel was being excreted by intestine in such cases, similar to the diarrhea that frequently follows metallic poisoning, such as arsenic. Drinker and others[17] observed that nickel ingested by human beings is largely excreted by intestine.

Fever was not a prominent symptom even in the critically ill patients. With few exceptions, the temperature of the sick men was not elevated above 101 F. Almost all of these had a moderate leukocytosis of 10,000 to 15,000 leukocytes per cubic millimeter. Hepatic and splenic tenderness was observed in several of the more critically ill patients, and several of them showed minimal jaundice. All of the hospitalized patients except the one who died four days after exposure received dimercaprol. The dimercaprol dosage was 2.5 mg. per kilogram of body weight every four hours for a total of six doses. Since the diagnosis of nickel carbonyl poisoning was not made until four days after exposure, none received dimercaprol earlier than the fifth day. It is our considered opinion that the administration of dimercaprol was beneficial in practically all cases and may have been lifesaving in several.

In practically all of the patients convalescence was protracted. Examination of a number of the hospitalized men (20 of the 31) two months after exposure revealed the presence of slight cyanosis in some of them, and many of them still had residual tenderness over the hepatic and splenic areas. Practically all of the men examined during convalescence complained of tiredness and unusual fatigue on exertion. They volunteered the opinion that they were unable to undertake their work with the same vigor as prior to the accident. Two months after exposure, most of the men had unusually high pulse rates. These persons included heavy boilermakers, whose normal pulse rates are frequently below 70 per minute. However, without exception, pulse rates of the 20 men examined were over 80 per minute, and, in 10 of them, the pulse rates were over 100.

Concentration of Nickel in Urine.—The normal concentration of nickel in urine is found to be 1.1 mcg.

15. Barnett, G. P.: Annual Report of the Chief Inspector of Factories for the Year 1948, London, Her Majesty's Stationery Office, 1949.

16. Drs. L. R. Byrd Jr. and A. B. Christie of Port Arthur, Texas, are preparing a detailed analysis of the clinical observations.

17. Drinker, K. R., and others: Hygenic Significance of Nickel, J. Industrial Hygiene 6: 307-356, 1924.

per 100 ml., standard deviation ± 0.85. Much higher values are observed after exposure to nickel carbonyl. Available information on nickel excretion in urine after accidental exposure is summarized in table 1. This table includes data for 17 subjects studied by us and 2 subjects studied by Bayer.[8] Patients in cases 1 to 4 inclusive were exposed at different times and locations; patients in cases 5 to 17 inclusive were part of the hospitalized group described in the previous section of this paper.

Data are given in table 1 for two patients (cases 1 and 2) who were subjected to slight exposure. In both cases specimens of urine collected during an eight hour period beginning shortly after exposure had nickel concentrations of over 10 mcg. per 100 ml. No symptoms, initial or delayed, were observed in either case. In case 2 it may be noted that the concentration of nickel in urine had dropped to the normal range three days after exposure. These results point to the probability that excess nickel in urine will indicate exposure to nickel carbonyl vapor at levels too low to cause discernible acute symptoms.

per 100 ml. with a maximum of 41. None of our values are as high as those reported by Bayer[8] on samples obtained post mortem (cases 18 and 19).

From these studies it is concluded that the concentration of nickel in urine is high for at least 24 hours after exposure to nickel carbonyl, and that this concentration can probably be correlated with severity of exposure. Five days after severe exposure the concentration of nickel in urine is still usually very high, but exceptions may be found; indeed these exceptions occurred in two of our most critically ill patients, one of whom died.

Effect of Dimercaprol Therapy.—It was observed during this study that dimercaprol therapy increases the concentration of nickel in urine after nickel carbonyl exposure. Data are available for three categories classified according to the time elapsing between exposure and the beginning of therapy. In the first category (case 20, fig. 1) therapy was started less than 24 hours after exposure. In the second category (cases 5 to 17 inclusive of table 1) therapy was delayed five days, while in the third category (17 cases) the patients received dimercaprol therapy beginning more than five days after exposure.

TABLE 1.—*Concentration of Nickel in Urine After Exposure to Nickel Carbonyl*

Case No.	Symptoms	Mcg. per 100 Ml. Time After Exposure (Days) 1	2	3	4	5	6	7	8	Reference
1	None	11	...	...	..	...	..	...	...	This work
2	None	18	...	2.2	..	...	..	...	...	This work
3	Mild (no lost time)	27	...	...	..	...	..	...	2.4	This work
4	Moderate (lost time)	35	9.0	...	..	...	..	6.1	1.8†	This work
5	Severe (required hospitalization)	..	...	...	..	2.3	..	...	...	This work
6	Severe (required hospitalization)	..	...	...	..	37.0	..	...	...	This work
7	Severe (required hospitalization)	..	...	...	..	4.8	..	...	...	This work
8	Severe (required hospitalization)	..	...	...	..	9.5	..	...	...	This work
9	Severe (required hospitalization)	..	...	...	..	2.8	..	...	...	This work
10	Severe (required hospitalization)	..	...	...	..	41.0	..	...	...	This work
11	Severe (required hospitalization)	..	...	...	..	30.0	..	...	...	This work
12	Severe (required hospitalization)	..	...	...	..	14.0	..	...	...	This work
13	Severe (required hospitalization)	..	...	...	..	31.0	..	...	...	This work
14	Severe (required hospitalization)	..	...	...	..	14.0	..	...	...	This work
15	Severe (required hospitalization)	..	...	...	..	14.0	..	...	...	This work
16	Very severe (required hospitalization)	..	...	...	..	1.0	..	...	...	This work
17	Fatal (13 days)	..	...	...	..	2.4	..	...	...	This work
18	Fatal (5 or 6 days)	..	...	...	..	52.0*	..	...	...	Bayer[8]
19	Fatal (4 days)	..	...	..	400*	...	..	...	...	Bayer[8]

* Postmortem specimen. † 13th day.

In case 3, the patient had only mild symptoms and lost no working time. In case 4, the patient had moderate symptoms and lost working time but did not require hospitalization. The concentration of nickel in the urine in both cases exceeded 25 mcg. per 100 ml. for specimens collected during the eight hours after exposure. In case 4 the value was still over 6 mcg. per 100 ml. of urine 7 days after exposure but dropped to within the normal range when the urine was examined 13 days after exposure.

Unfortunately no data on the excretion rate of nickel in urine in severe cases (5 to 17 inclusive) are available for the first four days after exposure since these cases were not diagnosed as nickel carbonyl poisoning until the fourth day. Nickel analyses in urine are not listed in table 1 for the period after the sixth day since the excretion of nickel was modified during this period by dimercaprol therapy (fig. 1).

In the severe cases (5 to 17 inclusive) that we studied, 12 out of 13 patients had values for nickel in urine above the normal range. The mean of these 12 cases is 17 mcg.

The only patient (case 20) who received prompt therapy was a chemist exposed during the course of research work. Acute effects were observed on the evening after exposure, and dimercaprol therapy was started the following day. Urine collected during the first day of dimercaprol administration was found to have nickel at a concentration of 1,900 mcg. per 100 ml. The concentration then returned rapidly to normal, as shown in figure 1. For this case, the nickel in the urine is equivalent to 60 ppm of nickel carbonyl vapor in 200 liters of air. This amount of air would be inhaled in about 30 minutes. The patient lost working time but did not require hospitalization.

Data for the 13 patients of the second group (cases 5 to 17 inclusive), are also presented in figure 1. The data show that dimercaprol therapy increased the concentration of nickel in urine in 10 of the 13 cases. The largest increases were observed in the severest cases. Concentrations of over 10 mcg. of nickel per 100 ml. were found in the urine of 12 of the 13 patients.

In the third group, the concentration of nickel in urine before the administration of dimercaprol ranged from 5 to 0.4 mcg. per 100 ml. with an average of 2.2 In most cases, significant increases did not occur after dimercaprol administration. In two cases, however, sharp increases in the concentration of nickel in urine were noted after therapy. In one case the increase was from 1.6 mcg. per 100 ml. of urine to 51 after therapy. The corresponding figures for the other patient were 1.8 and 29 mcg. per 100 ml.

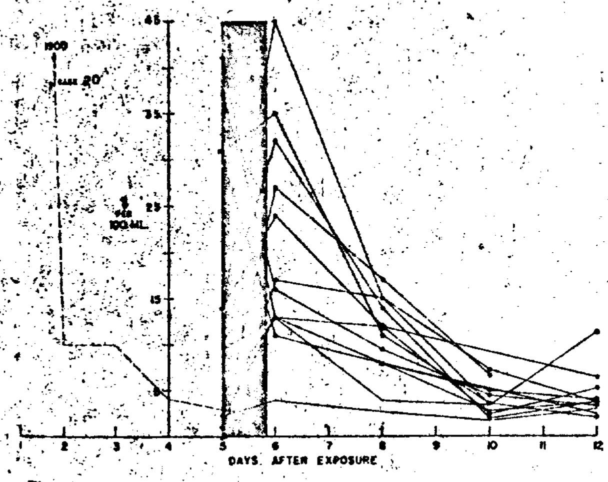

Fig. 1.—Nickel in the urine after dimercaprol therapy. Dimercaprol therapy was begun less than 24 hours after exposure in case 20; in the other cases, therapy was not begun until the fifth day because of delayed diagnosis. The shaded area represents the period during which dimercaprol was being administered.

Data available for three cases, (17 to 19 inclusive) are summarized in table 2. The high concentration of nickel in the lung and kidney tissues obtained in the two cases (18 and 19) reported by Bayer [8] can be compared to the low values for case 17 studied by us. These findings provide further evidence that dimercaprol aids in the elimination of nickel after nickel carbonyl exposure. No nickel was found in liver tissue in any of the three cases studied.

Table 2.—*Nickel Concentration in Tissues from Three Persons who Died After Nickel Carbonyl Exposure*

Case No.	Mcg. per 100 Gm. Wet Weight of Organ — Organs: Lung	Liver	Kidney	Reference
17	0.4	0	0.4	This work
18	...	0	55	Bayer [8]
19	22	0	106	Bayer [8]

Concentration of Nickel in Blood.—The concentration of nickel in oxalated blood was determined in cases 5 to 17 inclusive. Specimens collected on the fifth day after exposure, before dimercaprol therapy, were found to vary widely in nickel concentration. The values averaged 63 mcg. of nickel per 100 ml. of blood and ranged from 16 to 225. After dimercaprol therapy, the highest value was 33 mcg. per 100 ml.; the mean was 11. Individual values as well as the mean, decreased during therapy. The normal range of values for the concentration of nickel in blood is not well established, but a preliminary determination indicates that the mean value of 11 mcg. per 100 ml. is probably within the normal range.

Necropsy Findings in Case 17.—This patient, a muscular white man 29 years of age, a pipe fitter by occupation, died 13 days after exposure to nickel carbonyl. The details of the autopsy findings will not be given here.[16] The main pathological changes were found in the lungs, which contained many consolidated areas and only a small amount of aerating tissue. The pleura was thickened with subacute inflammation and edema. The lung tissue showed extensive deposition of fibrinoid material and infiltration of young fibrous tissue into the alveolar walls. This young tissue showed marked fibroblastic activity. In some areas the alveolar walls were only moderately thickened; however, in most places the pulmonary parenchyma was found to be practically consolidated with fibroblasts (fig. 2). In certain sections of the lungs large numbers of histiocytes were found in the alveolar spaces. These cells were laden with a granular brownish-black noniron staining pigment. The liver was essentially normal excepting for prominent Kupffer cells containing brownish-black noniron staining granules similar to those observed in the lungs. The adrenals appeared normal grossly and histologically. Except moderate perivascular edema, the brain sections appeared normal.

Fig. 2.—Low and high power fields of lung tissue showing marked fibrous tissue infiltration 13 days after exposure to nickel carbonyl. In large areas of the lungs the pulmonary parenchyma was practically consolidated by fibroblastic material.

COMMENT

The mechanism of the toxic action of nickel carbonyl has never been adequately explained. Amor [7] held the view that nickel carbonyl passed through the pulmonary epithelium unchanged. It is difficult, however, to reconcile Amor's view with the known physical and chemical properties of nickel carbonyl. Nickel carbonyl is reactive with a variety of nitrogen and phosphorous compounds as well as most oxidizing agents. These known properties suggest that it is probably reactive with biological materials.

A number of investigators have found no carboxyhemoglobin in blood samples obtained after exposure to nickel carbonyl. The fact that no carboxyhemoglobin has been found does not constitute evidence that nickel carbonyl does not decompose in the lungs to yield carbon monoxide. This may be illustrated by the following example: Nickel carbonyl in a concentration of 0.4 mg. per liter of air 30 minutes is lethal for mice and rats and, according to our studies, may be presumed to produce toxic symptoms in man. This exposure may be estimated to produce a concentration of carbon monoxide in blood of 0.6 vol. %. This estimate assumes normal values for the volume of air breathed in 30 minutes (200 liters) and for the total blood volume (8 liters). It also assumes the quantitative absorption and dissociation of the nickel carbonyl as well as the complete absorption of the released carbon monoxide. The estimated value of 0.6 vol. % is within the range of concentration observed in normal persons.

SUMMARY

Clinical observations are reported on 36 persons accidentally exposed to the vapors of nickel carbonyl. Two patients died and many of the others were critically ill. The concentrations of nickel in urine and blood were determined in samples obtained from exposed persons. Our studies indicate that the nickel concentrations in urine and blood are increased manyfold above normal after exposure. Increase in the concentration of nickel in urine may be correlated with the severity of exposure. Dimercaprol (BAL) was administered to 32 exposed persons, 31 of whom survived. Insofar as we are aware, this is the first report of the use of dimercaprol in the treatment of nickel carbonyl poisoning. The administration was attended by an increased excretion of nickel in urine and a marked decrease in the concentration of nickel in blood. It is our considered opinion that the administration of dimercaprol was beneficial in practically all cases and may have been lifesaving in several.

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220-223 MINERAL METABOLISM

Fed. Proc. 21(2)311 (1962)

223 MINERAL METABOLISM—Trace

Biochemistry

BINDING OF NICKEL AND OTHER TRACE METALS BY RIBONUCLEIC ACID.

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In previous studies from our laboratory, pulmonary carcinomas have been induced in rats exposed to the vapor of nickel carbonyl. As a continuation of these investigations, measurements were undertaken of nickel and other trace metals in ribonucleic acid isolated from tissues of normal rats and of rats exposed to nickel carbonyl. An ultra-violet spectrophotometric method was devised for the measurement of nickel in samples containing as little as 0.4 mcgm. Ni. Measurements of other trace metals were made by emission spectrography. Rat lung RNA was found to be rich in trace metals, and to contain nickel, chromium, lead, manganese, tin, and zinc in quantities which exceed those of RNA from rat liver, kidney and skeletal muscle by more than two-fold. Preliminary data indicate that nickel bound to lung and liver RNA is increased following exposure of rats to nickel carbonyl. (These investigations have been supported in part by contract with the Atomic Energy Commission.)

A.M.A. Arch. Indust. Health 20:36-41 (1959)

Nickel Poisoning

IX. Carcinogenesis in Rats Exposed to Nickel Carbonyl

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A preliminary report was made of our studies of chronic exposure of rats to nickel carbonyl.[1] It was pointed out in that report that the surviving animals would be retained and observed for tumor development. Observations on survivors during the subsequent 18 months are given in this present paper. In addition, we have included studies of rats that survived two or more years after a single, near-lethal exposure to nickel carbonyl.

The hazards from both acute and chronic exposures to nickel carbonyl have been described in several previous communications.[2-4] Attention has been directed to the high incidence of cancer of the respiratory tract among workers of long service in operations involving nickel carbonyl.[5-7] In our preliminary report [1] it was pointed out that rats chronically exposed to nickel carbonyl developed a remarkable degree of squamous metaplasia of the bronchial epithelium. It was noted that the degree of the metaplasia warranted continued investigation of nickel carbonyl as a possible carcinogenic agent.

Methods

The method of exposing rats to nickel carbonyl has been described previously.[1] In brief, test animals were exposed for 30 minutes, thrice weekly, throughout the first year of the experiment to concentrations of nickel carbonyl, given in the Table. It will be seen (Table) that at the end of the first year of our study only 14 control rats

Exposure Data

Rat Group	$Ni(CO)_4$ Dosage* Concentration, Mg/L.	Exposure	Initial No.	Deaths During Months† 0-12	13-24	25-3
C	0.0‡	3X weekly-1 year	41	27	11	3
X	0.03	3X weekly-1 year	64	48	11	5
Z	0.06	3X weekly-1 year	32	28	3	1
EP	0.25	single exposure	80	72	5	3

* Vaporized from a solution of $Ni(CO)_4$ dissolved in mixture of 50% alcohol and ether.
† Includes rats killed + spontaneous deaths.
‡ Exposed only to vaporized alcohol-ether vehicle.

(Group C) and 20 test rats (Groups X and Z) survived.

An additional group of 80 rats (EP) was exposed only once to nickel carbonyl in a concentration of 0.25 mg. per liter which approximates the LD_{50} value. Since the rats of Group EP had only one exposure (albeit a heavy one) to nickel carbonyl, our observations on this group were not included with those on the chronically exposed animals reported previously. They are given here for completeness in our study of long-term effects. In accordance with the design of the experiment the immediate mortality from this single, heavy exposure was high—52 of the 80 rats dying within the first week. Only eight animals were alive eight months after exposure.

At the end of the second year of observation the survivors included three control rats (C), six chronically exposed rats (X and Z), and three rats from the single, heavy exposure group (EP). The surviving animals died 24 to 30 months after starting the studies.

Results

Of the nine animals surviving two years or more after the initial exposure to nickel carbonyl, four were found to be tumor-bearers. One of these was from Group X (No. 23), one from Group Z (No. 21), and two from Group EP (No. 15 and No.

Received for publication Oct. 17, 1958.

This study was supported in part by contract of Jefferson Medical College with the Atomic Energy Commission.

From the Division of Metabolic Research, Jefferson Medical College, Rohm and Haas Company, and The Institute for Cancer Research.

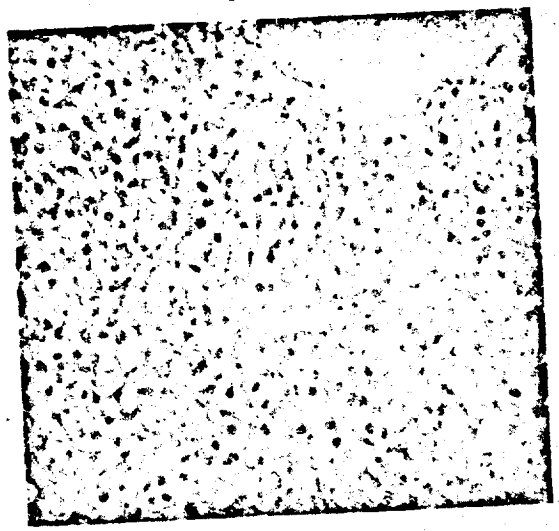

Fig. 1.—Carcinoma from lung of Rat Z-21. Grouping of clear cells here suggests nonacinar glandular origin, but elsewhere in the tumor a squamous-cell differentiation is simulated. × 180.

19). The first death of a rat bearing pulmonary tumors occurred 24 months after the first exposure to nickel carbonyl and 12 months after the last exposure. The deaths of the other three tumor-bearers occurred between 24 and 30 months after the initial exposure (Table).

In the lungs of the first tumor-bearer (Rat Z-21) the major disease consisted of multiple masses of clear-cell carcinoma having an adenocarcinomatous pattern (Fig. 1) but differentiating toward a squamous-cell type in some areas. Elsewhere in small areas of the lungs, a suspicious hyperchromatism of the lining cells in distorted alveoli (Fig. 2) resembled a stage of the adenomatoid transformation found by Hueper[8] in the lungs of guinea pigs exposed to powdered nickel. In this rat, metastatic tumor masses were also found in one kidney (Fig. 3) and near the great vessels of the heart.

The second rat found to have neoplastic disease in the lungs (Rat EP-15) died shortly after the first. This animal had been exposed to a single massive dose of nickel carbonyl two years before death. The pulmonary tumors were morphologically the same as those found in the first tumor-

Fig. 2.—Nuclear hyperchromatism in epithelial cells lining pulmonary alveoli distorted by fibrous thickening and papillations. May represent neoplastic or preneoplastic change in Rat Z-21. × 180.

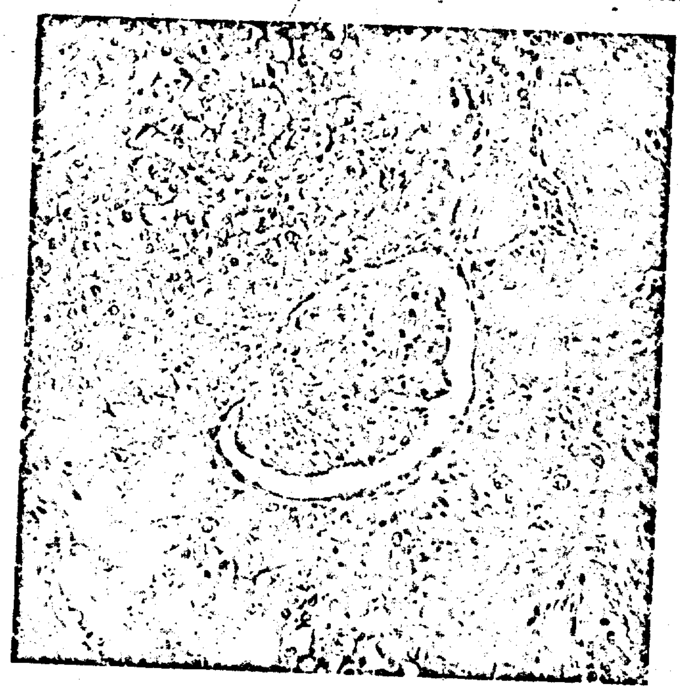

Fig. 3.—Metastatic carcinoma in kidney of Rat Z-21. Clear cells prompted an original suspicion of a renal origin, but additional studies indicated the respiratory system as the primary site. × 180.

bearer, but some areas gave evidence of more anaplastic malignancy (Fig. 4).

The last two rats bearing pulmonary tumors (Rat EP-19 and Rat X-23) had lesions less widely distributed in the lungs and morphologically different from those in the other two. Rat X-23 from the chronic exposure group died 27 months after the original exposure. The relatively small tumor in this rat consisted of moderately sized, slightly basophilic cells outlined by stroma in a fashion suggesting an alveolar spacing (Fig. 5). Two tumor thrombi were present in fairly thick-walled vessels adjacent to the main tumor. Classification of the lesion as a squamous-cell carcinoma is

Fig. 4.—Anaplastic carcinoma from lung of Rat EP-15. Elsewhere in the lung the clear-cell character of the tumor duplicates that in Rat Z-21. Rat had a single $Ni(CO)_4$ exposure two years before death. × 180.

guinea pigs and pulmonary adenomatoid proliferations in rats. The animals in Hueper's study were exposed six hours a day for four to five days per week for a maximal period of 21 months, at which time all of his animals had died. This is noteworthy, since the first neoplasms to appear in our rats were observed 24 months after the initial exposure to nickel carbonyl.

The reaction of nickel carbonyl on pulmonary tissue has not been extensively studied. Nickel carbonyl is reactive with a variety of nitrogen and phosphorus compounds and most oxidizing agents. It may be assumed to be reactive with most biologic materials. After acute exposure of experimental animals and man to nickel carbonyl, sections of lungs have been shown to contain many histiocytes in the alveolar spaces laden with granular, brownish-black, noniron-staining pigment. Similar granular pigment has also been observed in the Kupffer cells in the liver. It has not been established, however, as to whether or not these dark granules in our material represent metallic nickel or its compounds.

A number of investigators have found no carboxyhemoglobin in the blood of persons exposed to nickel carbonyl. It should be emphasized that these observations do not constitute evidence that nickel carbonyl is not dissociated in the lung to yield metallic nickel and carbon monoxide. It can be readily shown that the amount of carbon monoxide that would be released from exposure to lethal amounts of nickel carbonyl would be insufficient to elevate the concentration of carboxyhemoglobin above the range of concentrations observed in normal persons.

In the testing of inhalants for carcinogenicity the use of laboratory rats must be considered a severe challenge, since spontaneous pulmonary neoplasms occur only rarely in this species.[9-11] In our study the results give definite evidence of the carcinogenicity of nickel carbonyl for rat lung tissue. The incidence of carcinomas in our exposed animals is considered to be high in those rats surviving two years or more after exposure, but statistical analysis is not warranted on such small numbers.

Nickel and nickel carbonyl have been under suspicion as carcinogens for human beings for several decades,[5-7] and the present proof of carcinogenesis for the resistant rat lung adds evidence toward confirming these suspicions. In rats the carcinogenic potency is apparent even after a single inhalation exposure if they are observed during an adequate latent period.

Conclusions

Pulmonary cancers have been induced in rats subjected to a heavy, single exposure to nickel carbonyl as well as in rats subjected to repeated sublethal exposures for a period of a year. The cancers were not observed until two years or more after the initial exposure.

Our studies have shown that the inhalation of nickel carbonyl can cause pulmonary cancer in rats.

We wish to express our grateful appreciation to Dr. Shields Warren, Dr. Harold Stewart, and Dr. Thelma Dunn for reviewing our histologic sections and giving us the benefit of their opinions concerning the unprecedented nickel carbonyl-induced tumors in rats.

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Fig. 5.—Carcinoma from lung of Rat X-23 exposed to 0.03 mg. per liter of $Ni(CO)_4$ three times weekly for one year. Found at autopsy 27 months after original exposure. × 180.

warranted by the morphologic differentiation of some cell groups in that direction. No extrapulmonary neoplastic disease was discovered in this rat.

The last animal (Rat EP-19) remaining alive after a single massive exposure to nickel carbonyl was killed while in a moribund state 30 months after the exposure. At autopsy no pulmonary tumors were observed; however, histologic studies brought to light two small papillary bronchial adenomas (Fig. 6). These lesions were similar to the tumor found in a rat lung by Horn and Stewart.[9] *

* Drs. Harold Stewart and Thelma Dunn called our attention to this similarity.

Among the three surviving control animals exposed only to the vapors of the alcohol-ether solvent,† no pulmonary tumors developed. One control rat (Rat C-25) had a pheochromocytoma of the adrenal gland after 30 months of observation. This tumor has been considered to be spontaneous and unrelated to the control exposures.

Comment

Hueper[8] recently reported that the inhalation of finely powdered metallic nickel was associated with the production of benign and malignant pulmonary neoplasms in

† Nickel carbonyl is vaporized from a solution of $Ni(CO)_4$ dissolved in alcohol and ether.

Fig. 6.—Papillary adenomatous tumor from lung of Rat EP-19 exposed once to $Ni(CO)_4$ 30 months before death. One of two small pulmonary tumors. The other was peribronchial and less papillary in character. × 180.

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Recommended guideline for nickel (Ni) in drinking water Jan. 21, 1974

R. G. Tardiff, Research Toxicologist, CDB, WSRL

Gary Hutchinson, Chief, WSP, Region IV

The urgency of your need to have a guideline for nickel in drinking water has prompted me to make an evaluation and recommendation based upon the National Academy of Sciences recommendation to the National Aeronautics and Space Administration for manned space flights. The maximum allowable limit recommended for the long-term flights (i.e., 3 years) is 0.05 mg/l. The standard is based in part on the fact that the astronauts are very healthy individuals, and do not reflect the general public with respect to susceptibility to the toxic effects of chemical agents (that is, they are expected to be slightly less susceptible than the general population and mush less susceptible than special groups within the population).

In order to take into account the differences in individual susceptibilities, the limit could be lowered by one order of magnitude with assurance that a limit of 0.005 mg/l is reasonably safe. Newer information about the biological effects of nickel suggest that the above guidelines for Ni may be too conservative; however, until this newer information is fully evaluated, this limit of 5 μg/l should be applied for nickel in potable water. //

cc:
Mr. Tom P. Anderson
S. C. Dept. of Health
& Environmental Control

RGTardiff:dw:X8281

Arch. Mal. Prof. 26:263-264, 1965

New Experimental Research on the Toxicity of Nickel Powder introduced by the Digestive Route

Electrophoretic Changes in the Rabbit

by

M. Tardivel, P. Brunet-Antigny and E. Dervillée

Our experimental research was carried out on two series of rabbits poisoned over 9 months.

The first lot was composed of 10 animals to which we administered each day 5 to 20 mg/kg of pulverized nickel.

The animals were sacrificed at the end of this nine month period although they were found to be in perfect physical condition.

The second lot was also composed of 10 animals who received daily doses of pure nickel powder in amounts of 100 to 500 mg/kg of weight.

Contary to our previous research on guinea pigs, we obtained with rabbits subjected to massive doses a prolonged survival with good maintenance of general health during the whole duration of our experiemnt.

Results

Even though during the course of this experiment, we were not able to find clinical evidence of intolerance or poisoning, on the other hand, we regularly obtained, that is to say in 90% of the cases, very specific biological changes. The first series of animals was regularly followed from the hematological viewpoint. As in the poisoned guinea pigs, we noted a leucocytosis with lymphocytosis, varying from 9000 to 18000 leucocytes with an average of 60 to 80% lymphocytes.

Figure 1

Blood serum of the normal rabbit.

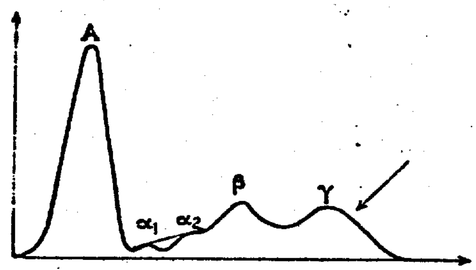
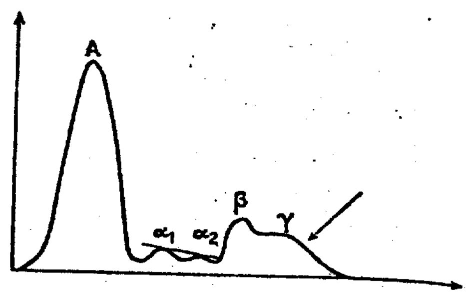


Figure 2

Blood serum of a rabbit poisoned with pulverized nickel (100 to 500 mg/kg) over a peroid of 9 months.



Besides the hematological study, on the animals of the second lot, we did an electrophoretic study of the serum

1) From the blood viewpoint, we found: a normal hematocrit, red blood cells quantitatively and qualitatively normal, a leucocytosis between 15,000 and 20,000 elements per cubic millimeter. The leucocyte formula is itself disturbed and comprises an average of 60% lymphocytes 15% monocytes with a certain number of lympho-monocytic cells already pointed out by Valerio (qualitative image of peripheric blood after experimental administration of nickel).

2) The electrophoretic study of the blood serum of poisoned animals shows profound changes in the level of the electrophoretic diagram. These changes were regularly found in all the animals of the experiment and effects exclusively the distribution of the globulins. The α 1 globulins are significantly increased while by contrast the α 2 and γ globulins are considerably decreased in proportions varying from 50 to 75% of their normal value. The β globulins can be statistically exploited.

In conclusion, the hematological and electrophoretic changes which we very consistently found in all the animals of the experiment were certainly related to the administration of nickel and enable us to say that this metal is likely to create within the organism certain biological problems. Still, it is necessary to specify whether this comes about because of a primitive and elective affliction of the serum proteins in the process of formation or because of a partial destruction of the fractions already elaborated or even whether we are here concerned with a secondary process corresponding to an injury of the formative tissues caused by nickel. The anatomic pathological examinations now in progress will perhaps enable us at the time of their conclusion to answer these hypotheses.

Arch. Mal. Prof. 26:263-4 (1965)

Nouvelles recherches expérimentales sur la toxicité de la poudre de nickel introduite par voie digestive. Modifications de l'électrophorèse chez le lapin.

Par M. TARDIVEL, P. BRUNET-ANTIGNY ET E. DERVILLÉE.

Nos recherches expérimentales ont porté sur deux séries de lapins intoxiqués pendant 9 mois.

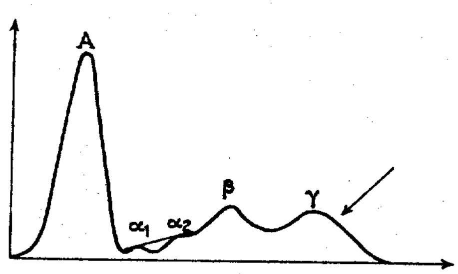
Le premier lot est composé de 10 animaux auxquels nous avons administré chaque jour de 5 à 20 mg/kg de nickel pulvérulent.

Les animaux ont été sacrifiés à l'issue de cette période de 9 mois, alors qu'ils se trouvaient en parfait état physique.

Le second lot comprend encore 10 animaux qui ont reçu quotidiennement des doses de nickel pur en poudre de l'ordre de 100 à 500 mg/kg de poids.

Contrairement à nos précédentes recherches sur le cobaye, nous avons obtenu chez le lapin soumis à ces doses massives une survie prolongée avec une bonne conservation de l'état général pendant toute la durée de notre expérimentation.

Résultats. — Si nous n'avons pu mettre en évidence, au cours de cette expérimentation, de manifestation clinique d'intolérance ou d'intoxication, par contre nous avons obtenu régulièrement, c'est-à-dire dans 90 p. 100 des cas, des modifications biologiques très spécifiques. La première série d'animaux a été suivie régulièrement au point de vue hématologique. Comme chez le cobaye intoxiqué, nous avons noté, une leucocytose avec lymphocytose, variant de 9 000 à 18 000 leucocytes avec en moyenne 60 à 80 p. 100 de lymphocytes.



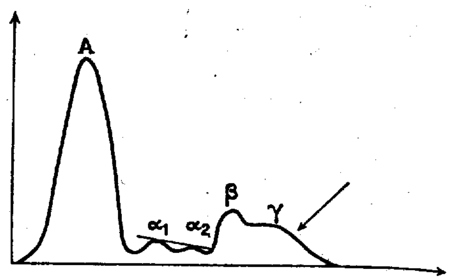


Fig. 1. — Sérum sanguin de lapin normal.
Fig. 2. — Sérum sanguin de lapin intoxiqué par nickel pulvérulent (100 à 500 mg/kg) pendant 9 mois.

Outre une surveillance hématologique, nous avons pratiqué chez les animaux du second lot, une étude électrophorétique du sérum.

1) Du point de vue sanguin, nous avons relevé : un hématocrite normal, des hématies quantitativement et qualitativement normales, une leucocytose se situant entre 15 000 et 20 000 éléments par millimètre cube. La formule leucocytaire est elle-même perturbée et comprend en moyenne 60 p. 100 de lymphocytes, 15 p. 100 de monocytes avec un certain nombre de cellules lympho-monocytaires déjà signalées par Valerio (image qualitative du sang périphérique après administration expérimentale de nickel).

2) L'étude électrophorétique du sérum sanguin des animaux intoxiqués fait ressortir de profondes modifications au niveau du diagramme électrophorétique. Ces perturbations se retrouvent régulièrement chez tous nos animaux d'expérience et portent exclusivement sur la répartition des globulines. Les globulines $\alpha 1$ sont sensiblement augmentées, par contre les globulines $\alpha 2$ et γ sont considérablement diminuées dans des proportions variant de 50 à 75 p. 100 de leur valeur normale. Les globulines β enfin ne subissent pratiquemeent pas de modifications susceptibles d'être exploitées statistiquement.

En conclusion, les modifications hématologiques et électrophorétiques que nous avons retrouvé d'une manière très régulière chez tous nos animaux d'expérience sont certainement en rapport avec l'administration de nickel et nous permettent de dire que ce métal est susceptible de créer au niveau de l'organisme certains troubles biologiques. Encore faudrait-il préciser s'il s'agit d'une atteinte primitive et élective au niveau des protéines sériques en voie de formation ou une destruction partielle des fractions déjà élaborées, ou bien, si nous nous trouvons en présence d'un processus secondaire correspondant à une atteinte par le nickel des tissus formateurs. Les examens anatomo-pathologiques en cours nous permettront peut-être, au moment de leur exploitation, de donner une réponse à ces hypothèses.

The Journal of Biological Chemistry
Vol. 234, No. 12, December 1959
Printed in U.S.A.
3257-3262

Nucleic Acids and Metals*†

I. CHROMIUM, MANGANESE, NICKEL, IRON, AND OTHER METALS IN RIBONUCLEIC ACID FROM DIVERSE BIOLOGICAL SOURCES

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(Received for publication, July 15, 1959)

The role of metals in the function and structure of many enzymes has been well documented (2). Their occurrence or possible role in the structure and function of nucleic acids has not been determined.

Metals have been known to interact with nucleic acids and their substituent nucleotides. There have been repeated, though sporadic reports, that nucleic acids, isolated from various sources actually contain or interact with one or several metals (3-17). Various elements, but particularly the alkaline earths, have also been found to effect both the structure and some functional characteristics of chromosomes (18-23). The concentration of magnesium in the medium in which cytoplasmic ribose nucleoprotein particles are suspended affects their structure, and the degree of polymerization of these particles depends on the magnesium concentration of the medium (24-27). The capacity of nickel, among other elements, to preserve the infectivity of tobacco mosaic virus ribonucleic acid has been commented upon (28). Although these studies have served to focus attention on the possible interaction between metals and nucleic acids, they have been either incomplete in terms of the compositional characterization of nucleic acids or inferential in the sense that the effect of *added* metals on nucleic acid or nucleoprotein structure has been examined.

In this first, systematic examination of nucleic acids isolated from a variety of tissues and species, they are shown to contain large amounts of firmly bound metals.

This examination of the metal content of nucleic acids resulted from a systematic search for a chromium-containing natural product, based on the similarity of the atomic structure of this metal to that of other elements of the first transition period of the periodic table. Vanadium, iron, cobalt, copper, and zinc have all been found to be part of biologically active, native compounds. Chromium, nickel, and manganese, although chemically very similar to those metals of the first transition group of the periodic table, had not been detected as part of such specific systems though such occurrence has been predicted (29).[1] This "transperiodic" analogy led to the isolation of a chromium containing substance from beef liver. This material proved to be a nucleoprotein which contained 1080 μg of chromium per g dry weight, representing a 20,000-fold aggregation of this metal, over the 0.05 μg per g, in whole liver. This substantial aggregation in a specific biological material is presently a unique observation and implies a specific association (31). This substance also contained large quantities of nickel, magnesium, calcium, strontium, barium, aluminum, iron, copper, and zinc. The knowledge of the presence of metals in nucleic acids suggests new approaches to the elucidation of the biological function and specificity of these compounds.

EXPERIMENTAL

Materials and Methods

RNA was obtained by the phenol extraction method (32, 33). All mammalian organs, *e.g.* beef liver, calf pancreas, calf thymus, and horse kidney, were obtained directly from a slaughter house, cooled immediately, and stored at $-20°$ until processed; they were then homogenized in a Waring Blendor with twice the volume of 0.05 M potassium phosphate buffer, pH 7.4; the homogenates were then filtered through a Saran screen and an equal volume of 90% aqueous phenol was added to perform the extraction. *Euglena gracilis* was grown in the dark in a defined medium (34), the cells were disrupted with a Logeman hand mill immediately after harvest and then extracted with phenol as above. Reticulocytes were obtained by cardiac puncture from rabbits treated for 1 week with phenylhydrazine. The cells were lysed by suspension of the washed, packed cells in metal-free water followed by extraction with phenol. A high molecular weight RNA was prepared from rat liver by the method of Laskov (35). Male rats, weighing 250 g, were fasted for 48 hours and killed by decapitation; the livers were removed and placed immediately in liquid nitrogen (Hisaw strain, Harvard Biological Laboratories). After removal from liquid nitrogen they were placed in a precooled porcelain mortar cleaned with acid (36), and ground to a fine powder. The powdered livers were mixed with equal volumes of 90% aqueous phenol and 10^{-4} M EDTA,[2] pH 8.0, using 600 ml of this mixture for every 100 g of liver. The mixture was then homogenized in a Potter-Elvehjem homogenizer cleaned with acid (36), and the resulting homogenate was treated exactly as described (35). There was no contact with stainless steel in either of the last two preparations. Supernatant RNA

* Supported by grants-in-aid from the National Institutes of Health, the Nutrition Foundation, and the Howard Hughes Medical Institute.

† A preliminary account has been presented (1).

[1] The detection of manganese in transphosphorylase of crayfish muscle is in need of confirmation (30).

[2] The abbreviations used are: EDTA, ethylenediaminetetraacetic acid; OP, 1,10-phenanthroline.

TABLE I

Emission spectrographic analysis of 90% phenol solution[a]

Metal content (expressed in μg/ml)[b]						
Mg	Ca	Sr	Ba	Al	Fe	Cu
0.15	1.1	0.013	0.055	0.025	0.09	0.072

[a] The following elements were searched for in all spectrographic analysis. Only those detected are listed: Mg, Ca, Sr, Ba, Pb, Al, Cr, Mn, Fe, Ni, Co, Zn, Mo, Cd, Sn, Ti, Cu.

[b] Not detected: Mn, Cr, Ni, Co, Zn, Cd.

TABLE II

Metal content of beef liver RNA[a]

Mg	500	Cr	86
Ca	930	Mn	81
Sr	26	Fe	370
Ba	99	Ni	63
Al	37	Cu	147
		Zn	291

[a] Values given as μg per g of RNA.

TABLE III

Repeatability of spectrographic analyses obtained on single sample of beef liver RNA

Analysis	Metal content[a]									
	Mg	Ca	Sr	Ba	Al	Cr	Mn	Fe	Ni	Zn
I	560	870	23	46	17	55	66	240	62	310
II	560	930	28	48	11	46	58	180	49	310
III	600	890	24	54	14	47	60	160	58	240
IV	700	870	27	54	12	50	65	140	73	280

[a] Values expressed as μg per g of RNA.

TABLE IV

Metal content of RNA from various sources (in μg per g of RNA)

Source	Mg	Ca	Sr	Ba	Pb	Al	Cr	Mn	Fe	Ni	Cu[a]	Zn	Cd
Calf pancreas	340	1500	75	94	120	34	140	66	230	130	[b]	620	[c]
(S-RNA)calf pancreas	620	710	18	180	[c]	78	18	29	230	18	[b]	200	[c]
Calf thymus	91	540	46	38	[c]	21	77	28	220	74	76	200	[c]
Horse kidney	280	500	15	56	52	180	400	95	870	44	[b]	170	20
Rabbit reticulocyte	980	2100	190	220	[c]	140	82	57	41	51	[b]	170	[c]
Euglena gracilis	400	920	27	91	69	32	76	73	180	60	190	650	[c]
Rat liver[d]	580	2000	14	81	[c]	630	102	33	180	64	[b]	1300	[c]

[a] Microchemical analysis.

[b] Not analyzed.

[c] Not detected.

[d] Procedure of (35).

of calf pancreas was prepared by phenol extraction of the "pH 5" precipitate of the supernatant fraction of calf pancreas.[3]

DNA was prepared from beef liver by extraction with 10% sodium chloride solution (37) and by phenol extraction (10, 38, 39).

[3] Kindly supplied by Dr. A. Meister, Tufts University, School of Medicine.

Metal-free water, prepared by ion exchange, was used throughout (36). All glassware was cleaned with acid before use, as previously described (36), to ensure against contamination. The reagent grade phenol used for extractions contained insignificant amounts of metals. Table I shows a representative emission spectrographic analysis of this reagent. All other reagents used in the extraction procedures were reagent grade and either were extracted with dithizone in carbon tetrachloride or passed over ion exchange beds of IR 120 resin (Rohm and Haas). Spectrographic analysis of all reagents after purification revealed no significant metal contamination.

RNA was measured by the orcinol method (40), DNA by the diphenylamine procedure (41), and protein by the Lowry phenol method (42). Samples were prepared for metal analysis by dry ashing in a quartz-lined muffle furnace at 700°. Emission spectrographic analyses were carried out with the use of the porous cup spark method previously described (43). Chromium was also measured microchemically with a modification of the diphenylcarbohydrazide method (44). Copper was measured with the sodium diethyldithiocarbamate procedure (45); cadmium was also determined by dithizone extraction in chloroform (46).

Dialyses were performed in cellophane dialysis tubing (Visking Corporation) which was treated before use (47).

A Beckman model DU spectrophotometer or a Cary recording spectrophotometer was employed for absorption spectrophotometry.

All reagent grade chelating agents were obtained from the following sources: ethylenediaminetetracetic acid (Bersworth Chemical Company), 1,10-phenanthroline (G. F. Smith Company), dithizone and sodium diethyldithiocarbamate (Eastman Organic Chemicals), 8-hydroxyquinoline (Baker Chemical Company).

RESULTS

The metal content of beef liver RNA is shown in Table II. It contains significant amounts of magnesium, calcium, strontium, barium, aluminum, chromium, manganese, iron, nickel, copper, and zinc. The repeatability of the spectrographic method and of the ashing procedure are documented in Table III, which shows four replicate spectrographic analyses of the same sample of beef liver RNA.

The metal content of RNA from pancreas, supernatant of pancreas, and the thymus of the calf, horse kidney, rabbit reticulocytes, *Euglena gracilis*, and rat liver are shown in Table IV. All of the metals present in beef liver RNA are also present in significant amounts in the RNA's from these phylogenetically diverse sources. The preparations from calf pancreas, horse kidney, and *Euglena gracilis* contain lead; it is absent from the others. Cadmium is only present in the RNA from horse kidney cortex; it is not detected in that from any other source.

The large number of phosphate groups of RNA constitute obvious loci on this molecule for potential interaction with metals; stoichiometry between metals and phosphate groups might therefore be expected. The moles of phosphate per g of RNA, however, are far in excess of the number of gram atoms of any one metal per gram RNA. Even the total sum of all metals present, ΣMe, expressed in gram atoms per gram of RNA, is far less than the moles of phosphate present. In fact the ratio ΣMe:phosphate, in total gram atoms of metal per mole phosphate $\times 10^{-2}$, is 1:50.

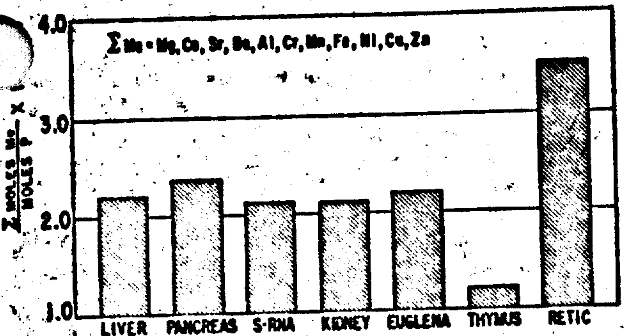

FIG. 1. The ratio of the sum of the micromoles of metals per gram of RNA to the phosphorus content per gram of RNA (ΣMe:P) in the RNA's from beef liver, calf pancreas, supernatant RNA of pancreas (S-RNA), horse kidney, *Euglena gracilis*, calf thymus, and rabbit reticulocytes. The data represent the mean of four replicate analyses, with the exception of beef liver RNA and pancreas RNA where 11 and 2 analyses were performed, respectively. The range of the ratios varied by <10% in all cases.

Internal, chance contamination of the phosphate group does not seem to account for the presence of metals in RNA. It would be expected that most of the phosphate groups would interact readily with the excess of existent metal ions if this phenomenon were due to such contamination. Furthermore, the ratio ΣMe:phosphate, although small, is remarkably constant regardless of the biological source of RNA. The ratio ΣMe: phosphate in the RNA's from beef liver, calf pancreas, supernatant of calf pancreas, horse kidney, and *Euglena gracilis* are [illegible]y similar, averaging 2.2×10^{-3} gram atoms of total metals [illegible]ole of RNA phosphorus. The only significant deviations were observed in the RNA from rabbit reticulocytes where the ratio is higher and in that from calf thymus where it is lower (Fig. 1).

Equally important is the observation that these metals are firmly bound to RNA, as evidenced by several experimental approaches. The metal content of all the various RNA's was not lowered by dialysis against large volumes of metal-free water, pH 5.8. Nor was the metal content affected significantly by dialysis of beef liver RNA against various buffers from pH 4 to 9 for 48 hours.

Further confirmation of the firm binding of metals to RNA was derived from dialysis experiments with several chelating agents. Aliquots of beef liver RNA were dialyzed for 48 hours at 4°, against 0.1 M EDTA, pH 7.0, 0.001 M 8-hydroxyquinoline, pH 7.0, and 0.001 M dithizone, pH 7.4. The results of dialysis for 48 hours against these three agents on the zinc, manganese, and iron contents of beef liver RNA are shown in Table V. EDTA and 8-hydroxyquinoline removed manganese and zinc, but the iron content is barely altered. Dithizone, which is poorly soluble in water, does not affect the metal content significantly under the conditions employed. In a further experiment, RNA was successively precipitated six times from 0.1 M EDTA by 3 volumes of 95% ethanol and then redissolved. Even after this exhaustive treatment significant quantities of strontium, barium, calcium, iron, chromium, manganese, aluminum, and magnesium were still present in this RNA (Table VI).

[illegible] specific and determined attempt to remove iron by dialysis [illegible]t (OP) was unsuccessful; when in excess, this agent forms [illegible]ghtly dissociable coordination complex with Fe^{++}, which (characteristically for this valence state (48)) absorbs maximally

TABLE V

Effect of dialysis with chelating agents on metal content of beef liver RNA

	Metal content[a]		
	Zn	Fe	Mn
Before dialysis	75	100	31
0.1 M EDTA[b]	0	71	0
0.001 M 8-Hydroxyquinoline[b]	0	96	14
0.001 M Dithizone[c]	71	89	27

[a] Values expressed as μg per g of RNA.
[b] pH 7.0.
[c] pH 7.4.

TABLE VI

Metal content[a] of beef liver RNA before and subsequent to six successive precipitations from solution of 0.1 M EDTA, pH 7.0

	Mg	Ca	Sr	Ba	Al	Cr	Mn	Fe	Zn
Control	370	260	49	37	27	12	24	390	50
EDTA treated (6 times)	8	210	4	3	13	14	8	140	0

[a] Values expressed as μg per g of RNA.

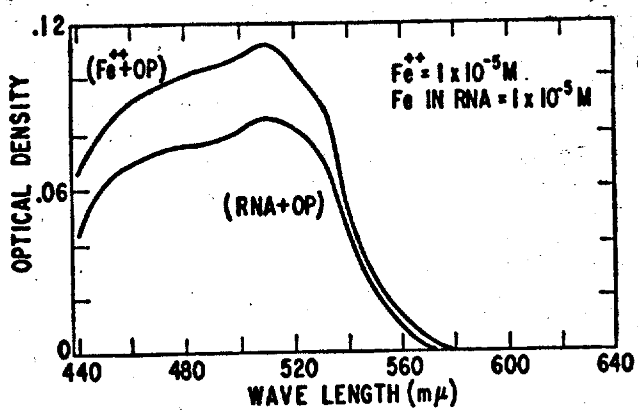

FIG. 2. Absorption spectra of 1×10^{-5} M Fe^{++}, the curve designated (Fe^{++} + OP) and RNA *containing* 1.5×10^{-5} M iron, (RNA + OP), in the presence of 5×10^{-3} M OP. The absorption spectra are virtually identical as a function of wave length with maxima at 510 mμ; the extinction coefficient of the RNA·OP complex at 510 mμ is less than that of the $[Fe(OP)_3]^{++}$ complex. Utilizing the extinction coefficient for the RNA·Fe·OP complex the iron in RNA can be determined directly, and is in agreement with measurements of iron after ashing of the complex.

at 510 mμ. Beef liver RNA (20 mg) dissolved in 2 ml of water was dialyzed against 200 ml of 5×10^{-3} M OP for 48 hours. The RNA solution within the dialysis bag noticeably absorbs light at 510 mμ and is visibly red. This specific absorption is not altered by repeated dialysis of this material against distilled water. Apparently, a mixed complex forms: RNA·Fe·OP. The absorption spectrum of an RNA solution containing 1.5×10^{-5} M iron, dialyzed against 5×10^{-3} M OP for 24 hours at pH 7, is shown in Fig. 2 (RNA·OP). The absorption spectrum of a solution of 1.5×10^{-5} M Fe^{++} to which 5×10^{-3} M OP has been added is also shown in Fig. 2 (Fe + OP). The absorption as a function of wave length of the two solutions is virtually identical

TABLE VII

Metal content[a] of beef liver DNA

	Mg	Ca	Sr	Ba	Al	Cr	Mn	Fe	Cu[b]	Zn
10% Sodium chloride extracted	10	140	[c]	1.9	75	150	[c]	330	68	110
Phenol extracted	110	280	21	110	140	81	25	400	[d]	120

[a] Expressed as μg per g.
[b] Microchemical analysis.
[c] Not detected.
[d] Not analyzed.

but the extinction coefficient of the RNA·Fe·OP complex is lower than that of the ionic complex. Such a change of the extinction coefficient may be indicative of, and is certainly consistent with, the presence of a *mixed complex* (49).

The metal content of DNA has also been examined. Table VII shows the metal content of DNA from beef liver, obtained both by phenol extraction and by extraction with 10% sodium chloride solution. In DNA the ratio ΣMe:phosphate in gram atoms per mole $\times 10^{-2}$ is distinctly less than that in RNA, amounting to only about one-third, or 1:150.

DISCUSSION

RNA, isolated from a wide variety of sources, extending from simple microorganisms to the biologically complex vertebrates, contained large amounts of different metals which were also found in ribonucleoprotein isolated by a different procedure. Chromium, manganese, and nickel, the transition elements of unknown biological association and function were present in every RNA examined. These elements have not heretofore been found to be aggregated to this extent in any other biological material; this supports the postulated specificity of interaction between metals and RNA. Since ribonucleic acids are known to be inhomogeneous, further purification may result in the isolation of ribonucleic acids which contain only *one specific* metal. A retrospective search of our analyses, performed during several years on many types of biological materials and recorded on some 400 spectrographic plates, has shown that these elements, although detected infrequently, were found primarily in materials known to have a high content of nucleic acids, *viz.* microsomes.

Metals were also found in DNA. However, the concentration of metals in terms of the total gram atoms of metals per nucleotide is much less in DNA than in RNA; this implies a specific interaction. If this finding were consequent to a spurious interaction with phosphate groups only, it might be expected that the ratio of metal to phosphate, *e.g.* of either type of nucleic acid, would tend to be identical.

The most obvious explanation for these findings, that they constitute an artifact as a result of external contamination, has been excluded by careful purification and analysis of the reagents and by rendering the equipment employed chemically sterile. The elaborate precautions developed in our laboratory for this purpose have been described previously (36). Stainless steel, as encountered in a Waring Blendor, does not contribute to contamination (36). Phenol, another obvious potential source of contamination, was analyzed and purified. Its metal content is insignificant and cannot account for these data; it did not contain Cr, Mn, or Ni, the elements singled out for inspection. Moreover, all these elements were encountered in the ribonucleoprotein, which was isolated in the absence of phenol (1).

Alternatively, spurious association of metals with RNA could result from circumstances other than biological association. Metals present in tissue, but either in ionic form or associated with another organic moiety (*e.g.* protein), could combine with RNA during the isolation procedure, a process designated *internal contamination* (36).

The phosphate groups of RNA would be likely sites for such spurious interactions. In spite of the large excess of metal ions present in tissues, the phosphate groups are far from saturated. Thus, *internal contamination* does not seem a likely explanation for these observations. However, these considerations do not eliminate the possibility of binding to phosphate groups, particularly since polyphosphates bind metals to a marked degree.

A cogent argument in favor of a specific association between the metals and RNA can be advanced from several lines of reasoning. Although the ratio of ΣMe:phosphate is not unity, it is quite constant (1:50) over a wide biological range of origin of RNA examined. This suggests a specific locus of interaction on the RNA molecule.

The formation of a coordination complex between metals and purine or pyrimidine bases deserves careful consideration. Although such a chelate complex has not been demonstrated unequivocally in nucleic acids, there is ample support for such a hypothesis. The nitrogens of nitrogenous bases, alone or in conjunction with their hydroxyl groups, constitute sites for the formation of chelates, for some of which dissociation constants have been determined (50–54). The keto-imide linkage of peptides has been suggested to interact with chromium to form very stable coordination complexes (55), and by analogy, the amide groups of these nitrogenous bases might be thought similarly to participate in metal binding. This suggestion, along with others, has been incorporated into models of the binding of magnesium to adenine and guanine (56). The recent detection of a hitherto unknown nucleoside, 5-ribosyluracil (57) for instance, implies that the organic composition of these substances is not known completely as yet and hence any suggestions advanced presently as to the nature of the binding site may have to be modified as new knowledge is gained.

The proposition, envisioning the formation of a coordination complex between metals and the nitrogenous bases or other covalent binding sites of nucleic acids, gains experimental support from the present studies which demonstrate very firm association, indeed, between metals and nucleic acids. All conventional procedures employed to remove loosely bound metals, *i.e.* dialysis, dialysis as a function of varying pH and with chelating agents, failed to remove all of the metals. In fact, subsequent to six successive precipitations of RNA out of a solution of 0.1 M EDTA, significant amounts of magnesium, calcium, strontium, zinc, aluminum, manganese, chromium, and iron are still present. Iron is bound so firmly that a mixed complex, RNA·Fe·OP is formed and iron 1,10-phenanthrolinate can be identified by its characteristic absorption spectrum. This circumstance yields approximations, at least, to the *intrinsic* dissociation constant of the RNA-Fe complex, since the constants for the $[Fe\ OP]^{++}$ complexes are known. The dissociation constant for the RNA·Fe complex is necessarily less than $K_1 = 1.6 \times 10^{-6}$, the dissociation constant of the $[Fe\ (OP)_1]^{++}$ complex, and may even be smaller than $K_3 = 8 \times 10^{-12}$, the cumulative dissociation constant of the $[Fe\ (OP)_3]^{++}$ complex. Thus, as has been pointed out elsewhere (47), the formation of a mixed complex of this type represents a first approximation to the determination of the intrinsic dissociation constant of a ligand-metal complex.

The presumable value of this constant for the RNA-Fe bond is higher by several orders of magnitude than that of an electrostatic bond, *viz.* metal-phosphate.

The extremely firm binding of metals to nucleic acids is reminiscent of a class of organometallic compounds first described by Kealy and Pauson who discovered and prepared biscyclopentadienyl iron (58, 59). Woodward *et al.* (60) have named this compound ferrocene. The appelation "sandwich complex" has been given to this type of metal interaction with resonating organic systems. Sandwich complexes are extremely stable and resist removal of the metal.

As early as 1921, Hein (61) reported unusual chromium salts of benzene, whose structure was finally described in 1956 by Fischer and Seus (62) in connection with their work on dibenzene chromium. A "mixed sandwich complex" between manganese, a substituted pentadiene, and benzene, has also been identified (63). In general these compounds require a resonating system with three π electrons from each of two aromatic molecules interacting with the 3rd orbital of the metal. Exceptionally stable complexes have been described for iron, chromium, nickel, and manganese, the elements here singled out for special attention. The pyrimidine and purine rings might be postulated to form sandwich complexes of this type, though experiment has not extended this hypothetical structure to these compounds. It is conceivable that metals may bring the nucleic acids into apposition through such a compound. Thus a purine-metal-purine, purine-metal-pyrimidine, pyrimidine-metal-pyrimidine "sandwich complex" could provide additional structural and configurational stabilization of nucleic acids, thought presently to be stabilized by hydrogen bonding primarily. Metals have in fact been shown to serve as crosslinking intermediates in the maintenance of the macromolecular structure of proteins as demonstrated by the action of zinc in maintaining amylase, alcohol dehydrogenase, and glutamic dehydrogenase in polymeric form (64–69). Chromium is particularly effective in serving as a crosslinking agent for collagen (55) and also for conarachin (70).

The presence of cadmium in the RNA of horse kidney is of particular interest since this metal is absent from the RNA of any other source.[4] An unusual protein containing 4% cadmium has been isolated from the cortex of the horse kidney (71, 72). The finding suggests that metals may be transferred from RNA to be incorporated into metalloproteins during their assembly and synthesis.

Metals might well serve to link the nucleic acid to the protein moiety of ribose nucleoprotein and such a suggestion has already been made for DNA on the basis of inferential data with chelating agents (38, 39).

Since the precise chemical function of nucleic acids is presently unknown these findings offer a new and unexpected parameter to explore this potential role. Nucleic acids, regardless of their source of origin, have a constant and relatively simple composition of purine and pyrimidine bases. This fact has formed the basis of the code, postulated to transmit and store information directing and orienting the biological specificity of other systems. The relative simplicity of composition of nucleic acids and the constancy of the ratios of their constituent nitrogenous bases, regardless of their source of origin, must result in a configuration presenting fewer degrees of freedom than that possessed by the amino acid arrangement in the protein for which the nucleic acids are presumed to carry the code (73).

The existent evidence to support the hypothesis that the *sequences* of nucleotides contain the properties which transmit and govern cellular specificity is inferential. There are at present no unequivocal chemical data which would break and decipher such a code (74). The new, compositional data described here must enter the considerations regarding the function of nucleic acids, such as the maintenance of macromolecular structure or the provision of specific sites of interaction between the nucleic acid templates of protein synthesis and amino acids, peptides, or proteins.

SUMMARY

Preparations of ribonucleic acid from phylogenetically diverse sources contain significant concentrations of metals. Chromium, nickel, and manganese, elements of the first transition group, not heretofore associated with specific biological compounds, are encountered regularly in concentrations several orders of magnitude higher than in the native material from which they are isolated. These and other metals are removed with difficulty by dialysis as a function of pH or by chelating agents. The iron of ribonucleic acid (RNA) of beef liver is shown to form a *mixed complex* with 1,10-phenanthroline (OP), RNA·Fe·OP, attesting to the firmness of attachment of this metal to this RNA. The ratio of the sum of the micromoles of all metals to phosphate of RNA, ΣMe:phosphate, is 1:50, whereas in DNA it is 1:150. Cadmium has been found only in RNA isolated from equine kidney, the source of a cadmium protein. The reproducible finding of large concentrations of many metals in RNA suggests that they may play a role in the maintenance of the configuration of the RNA molecule, perhaps linking purine or pyrimidine bases, or both, through covalent bonds possibly involving nitrogen atoms or π electrons of the bases. It is suggested that metals may bear a functional relationship to protein synthesis and the transmission of genetic information.

Acknowledgments—We are indebted to Dr. Ephraim Katchalski for many helpful discussions, to Dr. Keiichiro Fuwa for the experiments on removal of metals by ethylenediaminetetraacetic acid, and Miss Lois Hersum, Miss Flora Lerner, and Mrs. Bessie Tsokanis for excellent technical assistance.

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Arch. Biochem. & Biophys
51(1) 1-4 (1954)

The Metabolism of Nickel. I. Spatial and Temporal Distribution of Ni^{63} in the Mouse[1,2]

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Received January 20, 1954

Introduction

The precise role of nickel in the living organism is not known. The presence of this element has been detected in a variety of tissues of both plant and animal origin (1–5). The concentration of nickel in plants appears to be dependent upon the nickel content of the soil in which they have been grown (6). It is quite probable that the reported nickel content of animal tissues is derived from the vegetable food sources of the host and might possibly be considered as an artifact. Studies of the effect of Ni^{++} on various biological systems have been made and indicate Ni^{++} to activate arginase (7, 8), carboxylase (9, 10), and trypsin (11). Studies by Ohlmeyer (12) indicate Ni^{++} to inhibit acid phosphatase under certain conditions. The non-enzymatic decarboxylation of oxalacetic acid has been shown to be catalyzed by Ni^{++} as well as by other metals (10). Evidence has been presented showing that Ni^{++} can replace to a restricted degree the functions of Mn^{++} and Co^{++} in the activation of arginase (13), but the pH–activity curves have been shown to be different with each metal ion (14, 15). The reported toxicity of Ni^{++} to animals is probably due to inhibition of enzyme systems in important physiological centers (16, 17).

The reported high incidence of respiratory tract neoplasia and skin ailments among workers in nickel refineries (18–20), the widespread use of nickel compounds in the arts and industry, and the newly discovered

[1] These studies were sponsored by the Damon Runyon Memorial Fund for Cancer Research, Contract DRIR-230.

[2] Some of these data were presented at the 124th Meeting of the American Chemical Society, Chicago, September, 1953.

TABLE I

Distribution of Nickel in the Tissues of the Mouse Following the Administration of 1 μc. $Ni^{63}Cl_2$ (102 μg. Ni)

Counts per minute per gram tissue

Time	Lung	Kidney	Plasma	Red blood cells	Gastro-intestinal tract	Liver	Bladder	Brain	Heart	Spleen	Carcass residue[a]
hr.			*per ml.*								
2	39,445	75,007	11,283	5464	3801	6,264	2856	656	2206	4733	129
4	40,247	59,504	16,466	6358	1723	9,279	2800	821	4957	4353	294
8	19,130	70,438	5,601	7281	1798	10,430	1673	868	8124	1247	210
12	18,415	53,405	2,637	6104	3402	10,172	1269	1442	2275	3221	875
24	15,687	31,262	3,048	1687	1931	4,398	1339	428	1311	—	482
48	15,210	10,253	163	339	780	873	5287	208	626	435	506
72	15,568	5,608	62	192	—	704	3602	241	516	207	70

[a] Carcass residue includes muscle and bone after viscera and brain have been removed.

carcinogenic property of nickel (21) directed our attention to the study of the metabolism of Ni^{++} in the mammalian organism. The availability of high specific activity radioactive Ni^{63} with its long half-life of 87 years permitted the use of low nontoxic levels of the element in this investigation.

Experimental

Fifty-six adult male mice (C57 B1/6) weighing 16.4 ± 1.8 g. were administered 1 microcurie (μc.) $Ni^{63}Cl_2$ dissolved in physiological saline via intraperitoneal injection. Previous experimentation indicated this dose of Ni^{++} (102 μg.)[2] to be tolerated without noticeable toxic effects by this strain of mouse. The mice were housed in cages suitably arranged for collection of urine and feces. Samples consisting of eight mice were taken for analysis at 2, 4, 8, 12, 24, 48, and 72 hr. post injection. The mice were sacrificed by exsanguination via cardiac puncture. All tissues collected were washed free of adherent blood with ice-cold saline, blotted dry, and weighed. The entire organs were prepared for analysis. Wet digestion with HNO_3 and $HClO_4$ was employed. The tissue digests were taken to dryness 4–5 times, and the residues were given further treatment with $HClO_4$ until a pure white inorganic residue was obtained. The final residues were dissolved in dilute acid and transferred to volumetric flasks. Five-milliliter aliquots were taken for analysis, 0.5 mg. of nonradioactive Ni^{++} (as $NiCl_2$) was added as carrier, the solution was treated with 10 ml. of 0.75 M $(NH_4)_2SO_4$ in 7.5 M NH_4OH, and the Ni^{++} was separated by electrodeposition directly onto specially prepared copper

[2] The specific activity of the nickel used in this study was 1000 counts/min./0.075 μg.

TABLE II

Excretion of Nickel by the Mouse

Time after administration of Ni	Excretion rate	
	Urine	Feces
hr.	*counts/min./hr.*	*counts/min./hr.*
2	91	815
4	494	1402
8	104	8463
12	149	139
24	69	49
48	33	165
72	47	114

planchets. The operating voltage of the cells was 5 v. with 40 ma. current. The plated samples were assayed in a gas-flow detector, counting being continued to give a statistical standard error of less than 1%. Results are expressed as counts per minute per gram of tissue. All radio assay data have been corrected for decay, self-absorption, and coincidence. Recoveries of the total nickel administered to single animals were 98.2–99.8%. Details of the analytical techniques of separation of Ni^{63++} from biological material will be reported elsewhere.

Results

Maximal uptake of Ni^{63++} by all tissues was achieved in 2–12 hr. Kidney, lung, and plasma contained the highest concentrations of nickel; brain and muscle, the least (Table I). Most of the Ni^{63} was excreted via the feces in the first 8 hr.; urinary excretion of Ni^{63} was maximal in the first 4-hr. period (Table II).

Nickel disappeared rapidly from all tissues with the exception of the lungs and the brain. The lungs retained 38.6% of its uptake after 72 hr., the brain 16.7%. At the end of 72 hr. the ratio of concentration of tissue nickel to serum nickel was as follows: lungs, 250.6; kidney, 90.5; liver, 11.3; heart, 8.3; brain, 3.88; spleen, 3.34; erythrocytes, 3.08; and carcass, 1.13.

Discussion

The experimental data presented indicate Ni^{++} to be widely distributed and rapidly eliminated by the organism. The relatively high retention of nickel by pulmonary tissue suggests a high value for the complex-formation constant of Ni^{++} and lung protein. Whether this is due to some specific difference in lung protein composition is not yet known, but is being investigated in these laboratories. The data collected by Martell and Calvin (22) indicate metal complex-formation constants of heavy metals with amino acids, polypeptides, and other compounds to follow the order: $Hg^{++} > Zn^{++} > Cu^{++} > Ni^{++} > Co^{++} > Fe^{++} > Mn^{++}$. Thus it is probable that lung tissue will retain heavy-metal elements in this order also.

Since nickel has been shown to activate some enzymes and to inhibit others, it is not improbable that interference with these systems in the lung may impair catabolic processes involved in the elimination of the element.

Summary

1. Administered Ni^{63++} is widely distributed throughout the tissues and organs of the mouse. It is rapidly metabolized by the principal tissues with the exception of the lung and brain.

2. Nickel is rapidly eliminated via the feces and urine shortly after administration.

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metals with amino acids, polypeptides, and other compounds to follow the order: $Hg^{++} > Zn^{++} > Cu^{++} > Ni^{++} > Co^{++} > Fe^{++} > Mn^{++}$. Thus it is probable that lung tissue will retain heavy-metal elements in this order also.

Since nickel has been shown to activate some enzymes and to inhibit others, it is not improbable that interference with these systems in the lung may impair catabolic processes involved in the elimination of the element.

Summary

1. Administered Ni^{63++} is widely distributed throughout the tissues and organs of the mouse. It is rapidly metabolized by the principal tissues with the exception of the lung and brain.

2. Nickel is rapidly eliminated via the feces and urine shortly after administration.

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J. Anim. Sci. 28(5): 620-623

NICKEL TOXICITY IN YOUNG GROWING MICE [1]

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THE natural occurrence of nickel in both animal and plant tissue has been repeatedly demonstrated, although no direct evidence of a possible physiological role for nickel has been shown; *in vitro* studies have demonstrated that nickel activated arginase (Hellerman and Perkins, 1935), oxalacetic carboxylase (Speck, 1949) and trypsin (Sugai, 1944) systems. Nickel has also been shown to inhibit acid phosphatase (Ohlmeyer, 1946). The administration of Ni^{63} by intraperitoneal injection was found to result in a wide distribution of the isotope throughout mouse tissues (Wase *et al.*, 1954); the nickel was apparently metabolized rapidly by all tissues with the exception of lung and brain, and quickly excreted via feces and urine. In toxicity studies using rats, dietary levels of 250 to 1,000 ppm nickel did not affect growth rate or reproduction when fed for a 4-mo. period (Phatak and Patwardhah, 1950; 1952). Results of a later study suggested that a level of 250 ppm Ni did not influence either food intake or growth rate in young rats. Intraperitoneal injections of nickel were found to be lethal to white mice at low concentrations (Franz, 1962). These studies were initiated to determine the influence of toxic levels of nickel on feed utilization, growth, reproduction and the activity of several enzyme systems in mice.

Experimental Procedure

A Webster strain of Swiss mice supplied from the departmental colony was used in this experiment. The mice had access to feed and distilled-deionized water *ad libitum*, and were housed in stainless steel cages with raised wire floors at a room temperature of 24° ± 1° C.

The basal diet supplied all the required vitamins and minerals as established by the N.R.C. (1962) (table 1). Three dietary treatments were employed as follows: 0, 1,100 and 1,600 ppm of nickel added to the basal diet as the acetate salt.

In the first experiment, 12 mice (six males and six females) were used per dietary treatment for the 4 weeks of the study. The mice were housed with two mice of the same sex per cage and were weighed and sacrificed at the end of the fourth week of the experiment. Samples of liver, kidney and heart were obtained from 12 mice per dietary treatment, then frozen immediately, in a dry ice-acetone solution, and stored at −10° C. until analyzed for enzyme activities.

Isocitric dehydrogenase (Sigma, 1961), malic dehydrogenase (Sigma, 1957), succinic dehydrogenase (Cooperstein *et al.*, 1950), cytochrome oxidase (Cooperstein and Lazarow, 1951) and NADH cytochrome C reductase (De Bernard, 1957) enzyme activities were determined on the tissue sample homogenates. Bone citric acid levels were determined by the method of Hess and White (1955). In order to evaluate the effects of dietary treatments on the utilization of dietary ingredients, feed and fecal samples were collected during the last 2 weeks of the experiment and

TABLE 1. COMPOSITION OF DIET

Ingredient	% of diet
Ground yellow corn	20.00
Glucose monohydrate	19.75
Soybean meal	40.00
Fish meal	6.00
Dehy. alfalfa meal	1.00
Whey, dried	1.00
Corn distillers solubles	1.00
Corn oil	5.00
DL-methionine	0.20
Vitamin mixture [a]	2.50
Calcium carbonate	1.20
Dicalcium phosphate	1.90
Salt	0.20
Trace mineral mix [b]	0.05
Chromium oxide	0.20
Total	100.00

[a] Supplied the following per kg. of diet: (in milligrams) ascorbic acid, 12.5; thiamine·HCl, 12.5; niacin, 100.0; riboflavin, 20.0; pyridoxine·HCl, 12.5; d-biotin, 1.25; d-calcium pantothenate, 75.0; vitamin B_{12} (0.1%) 10.0; folic acid, 4.00; d-alpha-tocopheryl-acetate, 200.0; menadione (2-methyl-napthoquinone), 1.25; ethoxyquin, 500.0; i-inositol, 500.0; para-amino benzoic acid, 25.0; oxytetracycline, 25.0; (in IU) vitamin A, 4,500; vitamin D_2, 1,500 in a soybean meal carrier.

[b] Supplied the following as percent of diet: $Ca(OH)_2$, 0.8200; KH_2PO_4, 1.9760; NaCl, 0.5000; $MnSO_4 \cdot H_2O$, 0.336; $FeSO_4 \cdot 7H_2O$, 0.1320; $ZnSO_4$, 0.0240; $CuSO_4 \cdot 8H_2O$, 0.0020; KI, 0.0052; $CoCl_2 \cdot 6H_2O$, 0.0100; KCl, 0.5000; $MgSO_4 \cdot 7H_2O$, 0.5800; $H_2MoO_4 \cdot H_2O$, 0.003; KBr, 0.007.

[1] Arizona Agricultural Experiment Station Journal Article No. 1351.

TABLE 2. EFFECT OF NICKEL ACETATE ON GROWTH, FEED CONSUMPTION AND NICKEL INGESTED

Ni added, ppm	4-wk. wt./gm.		Feed consumed, gm./wk.		Ni ingested/ mouse, gm./wk.	
	Males	Females	Males	Females	Males	Females
0	24.2[a]	20.9[a]	34.8	28.7	T[c]	T
1100	23.2[a]	19.9[b]	32.1	26.5	35	29
1600	18.4[b]	17.5[b]	26.1	26.6	42	43

[a, b] Means within sex differ significantly ($P<.05$).
[c] Trace.

analyzed for Cr_2O_3 (Czarnocki *et al.*, 1961), calcium, phosphorus, protein, fat and gross energy (Weber and Reid, 1967). These were evaluated as "apparent" digestibilities as the ratio of feed to feces levels using the Cr_2O_3 marker. The data were analyzed statistically by the multiple range test; all differences were evaluated at the 0.05 level of probability (Duncan, 1955).

In a second experiment four pairs of weanling mice from the previous dietary treatment (0, 1,100 and 1,600 ppm Ni added) were weaned, matured and bred while maintained on the same diets. From these mice, the number of pups born and weaned was recorded per treatment. In addition, bodyweights of the adult mice were determined.

Results and Discussion

The feeding of diets high in nickel produced a significant reduction in growth when fed to young mice (table 2). In the first experiment, the fourth week body weights of male mice were unaffected by nickel levels of 1,100 ppm, but were significantly ($P<.05$) depressed by the 1,600 ppm level (table 2). The body weights of females were significantly ($P<.05$) depressed by either 1,100 or 1,600 ppm dietary nickel. The feed consumption of the males decreased with increased levels of dietary nickel, while feed consumption for the female mice remained virtually the same for all dietary treatments (table 2). The amount of ingested nickel, calculated from the feed consumption values, was found to be slightly less for females at the 1,100 ppm level than for males fed the same diet. These values were 35 mg. of nickel/week for males and 29 mg./week for females. The males and females ingested approximately the same amounts (42 mg. of nickel per week) when fed the 1,600 ppm diet. These results suggest that young growing female mice had a lesser tolerance level to nickel than males.

No marked changes in apparent digestibility of either energy, fat or protein were noted due to the feeding of nickel acetate (table 3). The work of Zipkin *et al.* (1963) indicated that bone citric acid levels in rats were decreased with the feeding of high dietary fluoride levels. It was, therefore, decided to determine bone citrate levels in this study to evaluate the effects of nickel on bone metabolism. The results of the first study showed no significant differences in bone citrate which could be attributed to the level of dietary nickel (table 4). Calcium and phosphorus metabolism were evaluated as the differences between dietary levels and fecal amounts in relation to the Cr_2O_3 marker and did not appear to be affected by dietary nickel (table 4). The average bone weights were not significantly altered in either males or females by the different levels of dietary nickel. These results would indicate that bone metabolism was not markedly affected by the ingestion of nickel.

A significant ($P<.05$) decrease was obtained in cytochrome oxidase and isocitric

TABLE 3. EFFECT OF NICKEL ACETATE ON PROTEIN, FAT AND GROSS ENERGY DIGESTION

Ni added, ppm	Apparent digestion coefficients					
	Fat		Protein		Energy	
	Males	Females	Males	Females	Males	Females
0	91.70	91.55	75.98	73.74	79.44	78.44
1100	91.66	93.10	72.80	78.12	75.57	82.12
1600	93.39	90.37	78.40	65.05	81.54	69.63

TABLE 4. EFFECT OF NICKEL ACETATE ON AMOUNTS OF CALCIUM AND PHOSPHORUS NOT RECOVERED IN FECES AND ON BONE CITRATE

Ni added, ppm	Bone citric acid, mcg./gm.		Ca apparently digested, %[a]		P apparently digested, %[a]	
	Males	Females	Males	Females	Males	Females
0	3.45	3.20	17.95	15.60	38.03	34.62
1100	3.39	3.49	17.80	17.96	31.70	36.05
1600	3.14	3.66	13.60	15.10	34.80	31.07

[a] Calculated as percent of ingested calcium and phosphorus not recovered in feces.

dehydrogenase activities of liver homogenates from mice fed either 1,100 or 1,600 ppm dietary Ni (table 5). In addition, NADH cytochrome C reductase activity of liver homogenates at 1,600 ppm dietary Ni was significantly ($P<.05$) decreased in activity. Cytochrome oxidase and malic dehydrogenase of heart homogenates were also significantly ($P<.05$) decreased in enzyme activity at the 1,600 ppm Ni level. In kidney homogenates, malic dehydrogenase was significantly ($P<.05$) decreased in activity at the high nickel level.

Significant differences in activities were not found for malic and succinic dehydrogenase in liver tissues and in cytochrome oxidase and isocitric dehydrogenase activities of kidney homogenates. Isocitric dehydrogenase activity of heart homogenates was not significantly changed by either the 1,100 or 1,600 ppm level of Ni (table 5). The results of the enzyme activity studies indicated that nickel exerted its influence in the kidney and liver where the element is known to concentrate. Nickel did not appear to affect any given enzyme system, but did decrease the activity in both the Krebs cycle and the electron transport systems.

TABLE 5. EFFECT OF NICKEL ACETATE ON THE ENZYME ACTIVITY OF BOTH MALE AND FEMALE MICE

Enzyme system[a]	Tissue	Dietary levels of nickel added: 0 ppm	1100 ppm	1600 ppm
Cytochrome oxidase	Liver	66^{b}	39^{c}	31^{c}
	Kidney	79^{b}	89^{b}	78^{b}
	Heart	50^{b}	43^{b}	21^{c}
Malic dehydrogenase	Liver	87^{b}	86^{b}	99^{b}
	Kidney	159^{b}	128^{b}	65^{c}
	Heart	112^{b}	111^{b}	95^{c}
Isocitric dehydrogenase	Liver	51^{b}	37^{c}	37^{c}
	Kidney	29^{b}	26^{b}	6^{b}
	Heart	42^{b}	40^{b}	30^{b}
Succinic dehydrogenase	Liver	57^{b}	42^{b}	54^{b}
	Kidney	4.4^{b}	3.1^{b}	0.8^{b}
NADH cytochrome C reductase	Liver	79^{b}	79^{b}	55^{c}

[a] Enzyme activity calculated as delta O.D./min./gm. of protein.

[b, c] Means with different superscript letters differ significantly ($P<.05$).

In the second study, male and female mice, weaned from the first experiment, were reared on the maternal dietary treatments. The effects of nickel on reproduction were examined. The results showed no effect of dietary nickel on mature body weights or on conception rate (table 6). The average number of pups born decreased with increasing levels of dietary nickel. The average number of pups weaned decreased significantly ($P<.05$) due to 1,600 ppm dietary nickel compared to the 1,100 ppm level and the basal diet (table 6). Females fed 1,600 ppm of nickel weaned an average of two pups while those fed 1,100 ppm or the basal diet weaned an average of seven pups. These results demonstrate that high levels of nickel had no influence on body weights of adult mice. The data on number of pups born and weaned did demonstrate a nickel toxicity influence.

Summary

The feeding of 1,600 ppm nickel to young growing mice resulted in growth reduction in both males and females. Female mice, in addition, showed a reduction in body weight gains when fed 1,100 ppm nickel. Nickel feeding did not exert a significant effect on apparent digestion coefficients for energy, fat and protein values. No changes in bone citrate values or in calcium and phosphorus utilization were observed. Liver enzyme activities for cytochrome oxidase and isocitric dehydrogenase

TABLE 6. EFFECT OF NICKEL ON NUMBER OF PUPS BORN AND WEANED

Ni added, ppm	Adult body wt., gm.		Av. no. pups born	Av. no. pups weaned
	Males	Females		
0	33.2	33.8	10	7^{a}
1100	31.5	32.8	9	7^{a}
1600	31.9	31.9	8	2^{b}

[a, b] Means with different superscript letters differ significantly ($P<.05$).

were significantly decreased in activity. The activity of malic dehydrogenase in kidney tissue was also significantly reduced. There was decreased activity in heart cytochrome oxidase and malic dehydrogenase. All other tissues tested for cytochrome oxidase, malic, isocitric and succinic dehydrogenase were unaffected. There was no significant effect of nickel ingestion on body weights of adult mice or on litter size but the number of pups weaned was reduced by 1,600 ppm of dietary nickel.

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J. Nutr. 95(4) 612-16 (1968)

Nickel Toxicity in Growing Chicks [1]

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ABSTRACT Two experiments have been conducted with chicks to determine the effects of high levels of dietary nickel on growth and nutrient utilization. Dietary nickel was supplied as either the acetate or sulfate salt at levels up to 1300 ppm in a basal diet calculated to be adequate in all known nutrients. Growth of chicks to 4 weeks of age was significantly depressed at 700 ppm nickel and above.

Metabolizable energy determinations on the respective experimental diets suggested an impairment in energy metabolism at the higher levels of dietary nickel. Fat retentions were not affected by nickel, but a marked reduction in nitrogen retention was obtained with the higher dietary levels of nickel.

In a second experiment, 1100 ppm nickel were incorporated into a basal diet and control diets pair-fed to delineate the effects associated with feed consumption and nickel toxicity per se. No significant differences in growth rate were obtained with either 1100 ppm nickel as the sulfate or acetate in comparison with the pair-fed controls. Nitrogen retention values were depressed in birds fed 1100 ppm nickel.

The effects of nickel have been clearly defined in rats, guinea pigs and mice. In a study with rats, it was found that levels of 250, 500 and 1000 ppm of nickel in three different forms did not significantly affect growth rate or reproduction (1). Approximately 71 to 91% of ingested Ni was found in the feces. Appreciable quantities were also retained in the tissues. In a further study, young rats fed on a diet containing 250 ppm of added Ni for 16 months grew normally (2). They found that maximum Ni levels occurred after 8 months and gradually decreased thereafter, probably because of decreased absorption of the nickel together with continued excretion. Low molar concentrations of nickel given to white mice by means of intraperitoneal injection were found to be lethal (3). Nickel given to guinea pigs by subcutaneous injections over a period of 120 days showed that the nickel was present in all organs investigated, and was eliminated primarily by the kidneys (4). Studies on the effect of Ni on various physiological systems have shown that Ni does activate arginase (5), carboxylase (6) and trypsin (7). Acid phosphatase under certain conditions was inhibited (8).

In view of the lack of information relating to the toxicity of nickel in poultry and the difficulties of extrapolating data from studies with rats and mice to chickens, the present study was initiated to evaluate the effects of high levels of this element on growth and the utilization of protein, fat and energy in the chick.

EXPERIMENTAL

Two studies were carried out with Hubbard broiler chicks grown to 4 weeks of age in batteries with raised wire floors. In the first experiment, nickel sulfate or nickel acetate was fed in amounts to supply zero, 100, 300, 500, 700, 900, 1100 and 1300 ppm added nickel to the basal diet. These experimental diets were supplied ad libitum.

In a second study, the 1100 ppm level of nickel was fed as the sulfate or acetate, and the basal diet was pair-fed with these diets to evaluate the separate effects of nickel on feed consumption and protein utilization. An additional treatment employed the basal diet fed ad libitum.

In each of the experiments, three replicate groups of 8 chicks each (4 males and 4 females) were fed each of the experimental diets. The basal diet (table 1) used in these studies was calculated to be adequate in all essential nutrients.

In addition to body weight gain and feed consumption data, feces samples

Received for publication November 13, 1967.

[1] Arizona Agricultural Experiment Station Journal Article no. 1288.

TABLE 1
Diet of nickel sulfate and acetate used in chick growth study

Ingredient	% of diet
sh meal	5.00
lfalfa meal (17% protein)	2.00
hey, dried	1.00
orn, diet, dried sol.	1.00
nimal fat	5.00
round yellow corn	49.50
oybean meal (44% protein)	31.15
icalcium phosphate	1.00
alcium carbonate	0.75
alt	0.20
langanese dioxide	0.02
L-Methionine	0.10
itamin mix [1]	2.50
hromium oxide	0.20
Total	100.00

[1] Supplied the following per kilogram of diet: (in mg) ascorbic acid, 12.5; thiamine·HCl, 12.5; niacin, 100.0; riboflavin, 20.0; pyridoxine·HCl, 12.5; *d*-biotin, .25; Ca D-pantothenate, 75.0; vitamin B_{12} (0.1%), 10.0; folic acid, 4.00; *d*-α-tocopheryl acetate, 200.0; menadione (2-methyl-naphthoquinone), 1.25; ethoxyquin, 500.0; *i*-inositol, 500.0; *p*-aminobenzoic acid, 5.0; oxytetracycline, 25.0; (in IU) vitamin A palmitate (stabilized), 14,000; and vitamin D_3, 1,500.

were collected during the fourth week of each experiment to determine the effects of the nickel on nitrogen, calcium, phosphorus and fat retentions and on dietary metabolizable energy values.

A 0.20% level of chromium oxide (Cr_2O_3) was mixed into the experimental rations as an inert marker and the ratio of this material to the analysis values obtained for the nutrients listed above was used to calculate retentions according to the formula:

% retention of "X" =

$$100 - 100 \frac{(Cr_2O_3 \text{ feed}}{(Cr_2O_3 \text{ feces}} \times \frac{\text{"X" feces)}}{\text{"X" feed)}}$$

Chromium oxide was determined on feed and feces samples by $HNO_3 - HClO_4$ digestion as outlined by Edwards and Gillis (9). The perchloric acid digest from the above determination was used for the determination of calcium by flame photometry employing a phosphorus correction (10) and for the determination of total phosphorus (11). The A.O.A.C. Kjeldahl method was used for nitrogen determinations while combustible energy values were determined with the Parr oxygen bomb calorimeter.

All data, where applicable, were subjected to statistical analysis by analysis of variance and the means separated by Duncan's multiple range test (12).

RESULTS AND DISCUSSION

No significant differences were obtained in the growth of chicks fed the two forms of nickel (tables 2 and 3). The birds fed nickel sulfate showed no significant differences in body weights up to the 300 ppm level, but the feeding of 500 to 700 ppm Ni significantly depressed weight gains in comparison with the control birds (table 2). A further progressive growth reduction occurred at the 900 to 1300 ppm levels of nickel. Feed conversion was not altered up to the 900 ppm level, at which point it was increased with each increasing level of nickel.

Nickel acetate gave results similar to those of nickel sulfate (table 3). No significant differences in body weights occurred up to the 500 ppm level but body weights were reduced at the 900 ppm level of nickel. Thus, nickel caused a progressive growth depression when fed as either the acetate or sulfate salts. Feed conversions were apparently not affected up to the 900 ppm nickel level but were depressed at both the 1300 ppm levels. Calculated amounts of nickel consumed per bird were quite similar for the two sources (tables 2 and 3). The 500 to 700 ppm levels appeared to be in a plateau region of intake. These results suggested that the amount of nickel ingested controlled the level of feed consumption. The amount of nickel ingested per bird, calculated as milligrams consumed per gram of gain, gave opposing data (tables 2 and 3). These data showed that as the birds ingested increasing amounts of nickel, body weight decreased in a direct relationship and that the effect of nickel was in addition to reduced feed intake. This problem was further examined in studying the metabolism of feed nutrients.

The effects of nickel on the utilization of dietary nutrients was investigated by means of Cr_2O_3 marker techniques. No great differences were found in metabolizable energy values or percent fat absorption with birds fed nickel sulfate (table 4). Metabolizable energy values were unal-

TABLE 2
Effect of dietary nickel sulfate on body weights, feed utilization and levels of nickel ingested in chicks

Nickel added as nickel sulfate	Body wt, 4 weeks	Feed conversion	Calculated nickel ingested to gain	Calculated nickel consumed per bird
ppm	*g*		*mg/g*	*mg*
0	565 [a,1]	1.78	T [2]	T
100	534 [a]	1.73	151	87
300	568 [a]	1.68	453	269
500	467 [ab]	1.69	687	406
700	376 [b]	1.97	794	412
900	247 [c]	2.11	837	396
1100	180 [c]	2.38	889	373
1300	179 [c]	2.82	1,347	478

[1] Means having different superscripts are statistically different at the 0.05 level of probability.
[2] T = trace.

TABLE 3
Effects of dietary nickel acetate on body weights, feed utilization and levels of nickel ingested in chicks

Nickel added as nickel acetate	Body wt, 4 weeks	Feed conversion	Calculated nickel ingested to gain	Calculated nickel consumed per bird
ppm	*g*		*mg/g*	*mg*
0	565 [a,1]	1.78	T [2]	T
100	514 [a]	1.79	152	85
300	559 [a]	1.66	429	259
500	484 [ab]	1.71	656	383
700	390 [b]	1.79	795	444
900	259 [c]	2.13	870	409
1100	256 [c]	2.04	1009	483
1300	173 [c]	2.54	1155	454

[1] Means having different superscripts are statistically different at the 0.05 level of probability.
[2] T = trace.

TABLE 4
Effect of nickel sulfate on the metabolism of some dietary nutrients

Nickel added	Gross energy retention	Metabolizable energy	Fat retention	Nitrogen retained	Protein efficiency ratio
ppm	*%*	*kcal/g feed*	*%*	*%*	
0	64.83	2.72	69.46	41.16	2.44
100	63.96	2.68	67.06	41.64	2.51
300	65.66	2.76	69.58	41.58	2.59
500	62.29	2.61	64.80	33.00	2.57
700	56.37	2.37	68.33	19.57	2.21
900	58.03	2.44	72.50	16.17	2.06
1100	56.06	2.35	75.75	12.50	1.83
1300	52.05	2.19	71.79	11.27	1.54

tered up to the 500 ppm level of nickel sulfate but appeared to be decreasing at levels above 500 ppm. Percent nitrogen retentions showed a decrease between the 300 to 500 ppm nickel levels. Nitrogen retentions dropped from 41% at the 300 ppm level to 33% at the 500 ppm level. It was further observed that as the level of nickel increased, the amount of nitrogen retained decreased. Protein efficiency ratios were found to decrease with higher levels of nickel sulfate. Above the 500

pm level of the diet a decrease occurred the PER with each increasing amount nickel fed above this level.

Nickel acetate, when compared with ckel sulfate, showed no significant alter- ions in either the gross energy or fat tained (table 5). The metabolizable ergy was unaffected by nickel acetate to the 1100 ppm Ni level. The feeding 1300 ppm Ni did not further lower the etabolizable energy figures. Percent ni- ogen retained was decreased by nickel etate feeding but no differences occurred til a 900 ppm Ni level was fed, and for ch increment added thereafter, a fur- er reduction occurred. The PER values nickel acetate were found to be slightly fferent from those of nickel sulfate in at a decrease did not occur until the 0 ppm Ni level was fed. A further re- ction in PER values occurred with gher levels of nickel acetate (table 5).

These results do not agree with the in vitro work of Sugai (7) who found an increase in trypsin activity with nickel. Our experimental data would suggest a reduction of proteolytic enzyme activity or increased protein catabolism. The question of whether the availability of nickel from the two sources was a factor in the differences noted between the sulfate and acetate salts or whether the sulfate ions were responsible for the differences in results noted cannot be resolved at this point.

In view of the apparent effects of dietary nickel on both feed consumption and nitrogen utilization, an additional experiment involving the feeding of 1100 ppm nickel was conducted. In this study, control groups were pair-fed at a level equivalent to the feed consumption of birds on the nickel-containing diets. When feed intake was equalized, there was no signifi-

TABLE 5
Effect of nickel acetate on the metabolism of some dietary nutrients

Nickel added	Gross energy retention	Metabolizable energy	Fat retention	Nitrogen retained	Protein efficiency ratio
ppm	*%*	*kcal/g feed*	*%*	*%*	
0	64.83	2.72	69.46	41.16	2.44
100	63.43	2.66	69.78	33.96	2.43
300	63.89	2.68	64.97	36.99	2.62
500	65.80	2.76	71.79	36.67	2.54
700	64.56	2.71	72.49	36.11	2.43
900	62.56	2.63	79.06	28.28	2.04
1100	58.33	2.45	73.03	20.34	2.08
1300	58.48	2.46	75.26	15.82	1.71

TABLE 6
Effect of dietary nickel on pair-fed chicks

Dietary treatment	Avg body wt 4 weeks	Feed consumed per bird	Feed conversion	Nitrogen retention
	g	*g*		*%*
1. Basal diet ad libitum	570 [a,1]	905	1.74	54.4
2. 1100 ppm Ni as nickel acetate ad libitum	304 [b]	538	2.12	44.9
3. Basal diet pair-fed with treatment 2	292 [b]	501	2.07	58.1
4. 1100 ppm Ni as nickel sulfate ad libitum	262 [b]	490	2.31	46.5
5. Basal diet pair-fed with treatment 4	259 [b]	451	2.16	63.4

[1] Means having different superscripts are statistically different at the 0.05 level of probability.

cant effect of dietary nickel on the growth rate of chicks to 4 weeks of age (table 6). No significant differences were noted between the acetate and sulfate salts. Feed conversions were slightly better in the pair-fed groups not receiving nickel. Nitrogen retentions were decreased with the feeding of 1100 ppm nickel, as either the acetate or sulfate, in comparison with the pair-fed control groups (table 6).

These data, along with the results obtained in the previous study, suggest that nickel, in addition to having an effect on feed intake, was detrimental to nitrogen retention. A striking difference in nitrogen retention values was obtained with nickel supplementation between the two experiments. The birds in the second study showed much higher nitrogen retention values at 1100 ppm nickel than in the first experiment. These differences are explained.

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A.M.A. Arch. Ind. Health
18: 480-2 (1958)

Nickel Poisoning

VI. A Note Concerning the Ineffectiveness of Edathamil Calcium-Disodium (Calcium Disodium Ethylenediaminetetraacetic Acid)

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Edathamil calcium-disodium (calcium disodium ethylenediaminetetraacetic acid, $CaNa_2EDTA$) has widespread application as a metal-complexing agent in a number of diversified fields. In the medical and biological fields it has been used as an antidote for lead poisoning [1-4]; it is reported to be effective in the treatment of cadmium [5] and uranium poisoning [6] in rats, in vanadium poisoning in mice,[7] and in the mobilization and removal of iron in patients with hemochromatosis.[8] Edathamil forms stable chelates with many metals, including nickel. When administered parenterally edathamil calcium-disodium is rapidly excreted in the urine.[9] Its toxicity * is of a relatively low order.[10]

Scudier and Tinazzi [11] reported edathamil to be an effective antidote for nickel chloride when administered intravenously in rabbits. No studies have been reported on the use of this agent in nickel carbonyl poisoning. Our studies were undertaken to determine the effect of edathamil calcium-disodium administered to experimental animals exposed to lethal concentrations of nickel carbonyl as well as to animals receiving lethal amounts of inorganic nickel salts parenterally.

Received for publication March 28, 1958.

From the Division of Metabolic Research, Jefferson Medical College, and Rohm and Haas Company.

This study was supported in part by contract of Jefferson Medical College with the Atomic Energy Commission.

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* L. D_{50} value is 500 to 700 mg. per kilogram of body weight, depending upon species and route of administration.

Results of Experiments

Effect of Edathamil in Mice Exposed to Nickel Carbonyl.—Four experiments were undertaken to evaluate the effect of various dosages of edathamil upon mice exposed to nickel carbonyl. In each experiment 20 mice were exposed to the vapors of nickel carbonyl in a concentration of 0.06 mg. per liter for a period of 30 minutes.[12] After exposure, 10 mice from each group received edathamil calcium-disodium and the remaining 10 served as controls. The dosages of edathamil calcium-disodium are given in Table 1. It will be seen that the mortality ratios in the groups receiving edathamil did not differ significantly from those of the control groups.

Postmortem examinations were made on some of the animals, and the amounts of

TABLE 1.—*Effect of Edathamil Calcium-Disodium on Mice Exposed to Nickel Carbonyl, 0.06 Milligrams per Liter*

Groups	$CaNa_2EDTA$ Dosage, Mg/Kg.	$CaNa_2EDTA$ Frequency of Administration	Mortality Ratios (3 Days) Dead/Treated
$Ni(CO)_4$			6/10
$Ni(CO)_4+CaNa_2EDTA$	40	2 doses daily—3 days	6/10
$Ni(CO)_4$			10/10
$Ni(CO)_4+CaNa_2EDTA$	200	2 doses daily—2 days	9/10
$Ni(CO)_4$			9/10
$Ni(CO)_4+CaNa_2EDTA$	500	2 doses—first day	10/10
$Ni(CO)_4$			8/10
$Ni(CO)_4+CaNa_2EDTA$	500	3 doses—first day	6/10

TABLE 2.—*Distribution of Nickel in Mice*

Group	Lungs	Kidney	Liver
	(Ni—µg. per Gm.)		
$Ni(CO)_4$ (Control)	9.12±2.89	10.03±2.55	2.06±0.41
$Ni(CO)_4$+$CaNa_2EDTA$	8.23±1.78	11.01±2.74	5.09±2.11

nickel contained in their lungs, liver, and kidneys were determined.[13] The results of these analyses are given in Table 2. No significant differences were observed between the distribution of nickel in the vital organs of the untreated animals and those treated with edathamil calcium-disodium.

Effect of Edathamil in Rabbits Exposed to Nickel Carbonyl.—Seven healthy male albino rabbits, weighing approximately 2.5 kg. and maintained on a diet of Purina Rabbit Chow Checkers, were used for these studies. Rabbits 1 and 2 were exposed simultaneously to nickel carbonyl in a concentration of 2.0 mg. per liter for 30 minutes. Rabbit 2 received an intravenous injection of edathamil calcium-disodium in a dosage of 500 mg. per kilogram of body weight immediately after removal from the exposure chamber. Rabbits 3 and 4 were exposed simultaneously to nickel carbonyl in a concentration of 1.75 mg. per liter for 30 minutes. Rabbit 4 received an intravenous injection of edathamil calcium-disodium in a dosage of 500 mg. per kilogram immediately after removal from the exposure chamber and 250 mg. per kilogram intravenously 18 hours later. Rabbits 5, 6, and 7 were also exposed simultaneously to nickel carbonyl in a concentration of 1.75 mg. per liter for 30 minutes. Rabbits 6 and 7 were given a solution of edathamil calcium-disodium intravenously in a dosage of 500 mg. per kilogram of body weight immediately after removal from the exposure chamber. Four hours later these two animals each received edathamil calcium-disodium intravenously in a dosage of 250 mg. per kilogram of body weight. All of the rabbits (control and treated) died within 90 hours after exposure to nickel carbonyl.

TABLE 3.—*Distribution of Nickel in Rabbits*

Rabbit *		Lungs	Liver	Kidney	Brain
		(Ni—µg. per Gm.)			
1	$Ni(CO)_4$ (Control)	10.87	1.78	12.12	
2	$Ni(CO)_4$+$CaNa_2EDTA$	12.01	3.68	4.94	
5	$Ni(CO)_4$ (Control)	12.73	6.08	5.15	3.14
6	$Ni(CO)_4$+$CaNa_2EDTA$	13.07	3.87	6.20	3.85
	24-Hour Urine (Ni—µg/100 Ml.)				
3	$Ni(CO)_4$ (Control)		1638.0		
4	$Ni(CO)_4$+$CaNa_2EDTA$		1506.0		

* See text for explanation of rabbit numbers.

Analyses for nickel were made on the lungs, liver, kidneys, and brains of Rabbits 1, 2, 5, and 6. The amounts of nickel were measured in 24-hour collections of urine from Rabbits 3 and 4 following exposure. It will be seen in Table 3 that there is no significant difference between the distribution of nickel in the organs of the control animals and those receiving edathamil calcium-disodium after exposure. Moreover, the amounts of nickel excreted in the urines of treated and untreated rabbits were essentially the same.

TABLE 4.—*Effect of Edathamil Calcium-Disodium After Intraperitoneal Injection of Nickel in Mice* (0.01% w/v Nickel Nitrate)

Nickel Dosage Mg/Kg.	$CaNa_2EDTA$ Dosage Mg/Kg.	Mortality Ratios (3 Days) Dead/Treated
0.5		2/10
1.0		5/10
1.5		7/10
2.0		9/10
2.5		10/10
2.0	100	10/10
2.0	250	10/10
2.0	500	10/10
1.0	250	8/10
1.0	500	8/10

TABLE 5.—*Effect of Edathamil Calcium-Disodium After Intraperitoneal Injection of Nickel in Rats* (0.1% w/v Nickel Nitrate)

Nickel Dosage Mg/Kg.	$CaNa_2EDTA$ Dosage Mg/Kg.	Mortality Ratios (3 Days) Dead/Treated
1.0		10/10
1.0	100	10/10
1.0		9/10
1.0	500	8/10

Effect of Edathamil on Mice and Rats Given Lethal Doses of Inorganic Nickel Salts.—Swiss albino mice weighing 18 to 20 gm. were fasted overnight prior to the intraperitoneal injection of a 0.01% (w/v) solution of nickel nitrate in dosages ranging from 0.5 to 2.5 mg. per kilogram of body weight. Fifteen minutes after the injection of the nickel solution, edathamil calcium-disodium was administered intraperitoneally in dosages of 100 to 500 mg. per kilogram of body weight. A similar experiment was also performed utilizing albino rats of the Wistar strain.

The results of these studies are presented in Tables 4 and 5. It will be seen that edathamil calcium-disodium in dosages as high as 500 mg. per kilogram of body weight does not provide protection against the toxic effects of nickel nitrate given parenterally to mice and rats.

Summary

Edathamil calcium-disodium (calcium disodium ethylenediaminetetraacetic acid) administered parenterally to mice and rabbits in dosages as high as 500 mg. per kilogram of body weight did not overcome the lethal effects of exposure to nickel carbonyl. Moreover, edathamil did not alter the distribution of nickel in lungs, liver, and kidneys of exposed animals and did not increase the amount of nickel excreted in the urine.

Edathamil calcium-disodium was found to be ineffective as an antidote in experimental animals receiving lethal amounts of inorganic nickel salts parenterally.

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